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Frequency of Concentrate Supplementation for Cattle Fed Barley Straw

by

Ruth Cara Tellier 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Masters of Science

in

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ***Frequency of Concentrate Supplementation for Cattle Fed Barley Straw***, submitted by Ruth Cara Tellier, in partial fulfillment of the requirements for the degree of Masters of Science in Animal Science.

Abstract

Five ruminally cannulated crossbred steers (465 ± 30kg) were fed diets containing 70% barley straw in a 5 x 5 Latin square design experiment to investigate the effects of frequency of feeding concentrate (daily, alternate days or every third day) and concentrate protein concentration (14.1 and 24.6%) on voluntary feed intake, ruminal dry matter disappearance, digestibility and rumen metabolite concentrations. Frequency of concentrate feeding had no influence on straw intake, total intake, disappearance of ruminally-incubated straw, or ruminal ammonia and lactate concentrations. Supplemental protein increased ($P < 0.05$) mean ruminal ammonia concentrations (3.3 vs. 1.6 mM), 24 h ruminal straw disappearance (41.1 vs. 37.5%), and in vivo dry matter digestibility (57.5 vs. 54.7%), but had no influence on voluntary dry matter intake (7.87 kg d^{-1}). It was concluded that concentrate can be fed every second day without any negative impact on parameters which were measured. However, more research is required to study the feasibility of feeding concentrate every third day.

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CHAPTER 1

Introduction

Alberta boasts over 40% of the national cow-calf herd with approximately 2.8 million head in 2000 (Statistics Canada 2001). The majority of these cattle are fed conserved forages during the winter. Winter feeding costs are the single greatest cost of production for most cow-calf operations. These costs average approximately 33%, but can range from 17.4% to 47.7% of total production costs ($21.00 to $60.00 per lb of calf weaned) depending on where the producer is situated and the year (AAFRD 1999). As a result, there has been great attention paid to finding ways of reducing winter feed costs as a mean of increasing profitability of cow-calf enterprises.

One way of reducing feed costs is by feeding low quality forage and byproducts of cereal production (i.e. cereal straw). Cereal straw is generally cheaper than hay and has been shown to have great feeding potential for dry, gestating cows. Production of wheat, barley and oats in Alberta in 2000 was over 13.3 million tonnes (AAFRD 2001). Using the kg yield of residue per kg grain produced (wheat 0.9 kg; barley 0.64 kg; oats 0.9 kg) used by AAFRD (1999) there would potentially be 10 million tonnes of straw available for animal consumption. There would be enough straw to feed approximately 5.6 million head of cattle (590 kg; consuming 1.5% body weight) for 200. That is 2 times the Alberta beef cowherd.

The concerns with feeding straw are low voluntary intake, low protein content, poor digestibility, and slow passage rate. As a result, supplementation with concentrate or good quality hay is required to maintain performance of the beef cow. There is interest in studying the effects of feeding supplements less frequently than once daily to

help reduce production costs. Reduced costs would be realized through decreased labour requirements and possibly machinery expenses such as repairs and fuel costs.

Frequency of supplementation of straw-based diets has not been studied extensively. The school of thought is that by providing a consistent diet daily the rumen environment will not experience large fluctuations therefore providing a more hospitable environment for microbial production. Recent research by Huston et al. (1999) found that supplement and forage intake was more variable within groups fed supplement once daily compared to those fed a supplement less frequently. Reducing supplementation frequency did not significantly impact the rumen environment and ruminal metabolism in the studies of Chase and Hibberd (1989), Collins and Pritchard (1992), and Beaty et al. (1994). Digestibility of protein, dry matter (DM) and organic matter (OM) were not affected by supplementation frequency (Coleman and Wyatt 1982; Chase and Hibberd 1989; Brandyberry et al. 1992; Collins and Pritchard 1992). In fact, Beaty et al. (1994) found that reducing supplementation frequency from once daily to three times per week increased DM and neutral detergent fibre digestion (NDF).

One concern with feeding less frequently is that production may be negatively impacted. The results have been variable. McIlvain and Shoop (1962) found no difference in live weight gain between steers fed cottonseed cake daily or every third day. Pate and Crockett (1971) found that steers fed a high protein supplement every third day actually gained more compared to those fed the same supplement daily, and Beaty et al. (1994) found that decreasing the supplementation frequency increased winter weight loss of grazing cows.

It is important to investigate new methods of reducing feed and production costs to try to increase profitability of the cow-calf enterprise. Reducing supplementation

frequency is a very promising way of reducing costs. More information on how supplementation frequency impacts the rumen environment and metabolism is required to determine if it is a feasible management option for the cow-calf producer.

1.1 Objectives

The hypotheses for this experiment were that decreasing supplementation frequency will not negatively impact voluntary feed intake, straw degradability, diet digestibility or the rumen environment, and that supplemental protein would influence the above parametrs and is required with straw-based diets. The specific objectives were to compare the following treatments: 1) feeding a low protein supplement daily, 2) feeding a low protein supplement every other day, 3) feeding a high protein supplement daily, 4) feeding a high protein supplement every other day, and 5) feeding a high protein supplement every third day.

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CHAPTER 2

Review of the Literature[1]

2.1 Cereal Straw in Diets of Wintering Beef Cows

In Alberta, there was approximately 13.3 million tonnes (AAFRD 2001) of wheat, barley, and oats grown in 2000. There would be enough straw available to feed almost two times the Alberta cow-calf herd for 6 months. Cereal straw as a feed resource has become of more interest to producers seeking to minimize their winter feeding costs.

Weisenberger et al. (1976) found that there was little difference in the liveweight gain of cows fed 78, 86, or 94% straw plus a protein supplement. In a later study by Mathison (1976) cows were fed 85 to 95% straw plus a barley, rapeseed meal, or urea supplement. Those cows fed 95% baled or chopped straw or 100% straw lost 36, 49, and 42 kg body weight respectively. Body weight changes in cows fed 85% straw ranged between -14 to +6 kg.

Kay et al. (1968) found that cows fed barley straw (9.2, 7.5, 8.5 kg daily respectively) supplemented with a urea/maize meal block, barley grain (2 kg d^{-1}), or a volatile fatty acid (VFA) solution lost 0.27, 0.19, and 0.44 kg of body weight per day. There were no calving difficulties and within 10 weeks after calving, the cows had regained the weight lost over the trial period. Results were similar in a second trial where the cows were fed straw with barley, or a barley/urea supplement.

Males et al. (1982) found that cows (460-480 kg liveweight) wintered on diets containing 67 or 75% wheat straw had a daily requirement of at least 0.65 kg of crude protein.

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[1] Abbreviations: **ADF** = acid detergent fibre; **DM** = dry matter; **NDF** = neutral detergent fibre; **NPN** = nonprotein nitrogen; **VFA** = volatile fatty acid

Limitations to feeding cereal straw includes its low protein content, low voluntary intake, low digestibility, and slow passage rate through the gastrointestinal tract. Some of these limitations can be overcome by chemically or physically processing the straw, or by protein and energy supplementation. Many of these factors are discussed in this review.

2.2 Factors Influencing Voluntary Intake of Straw-based Diets

Intake influences the consumption of nutrients required for production. One of the objectives of this experiment was to determine the effect of supplementation frequency on the voluntary intake of straw. Therefore, it is necessary to understand the factors that impact the consumption of feed by cattle.

There are many factors affecting intake, including animal, environmental, and diet factors. Animal factors include rumen fill, clearance of undigested particles, palatability, body composition, gender, age when placed on feed, breed, and physiological state. Diet factors include, but are not limited to, forage quality, forage availability in grazing animals, physical and chemical processing of the feeds, nutrient deficiencies, energy supplementation, protein supplementation, and various management factors. Management factors include the use of growth promotants, and the use of feed additives.

2.2.1 Animal Factors Affecting Intake

2.2.1.1 Rumen Fill

The mass and volume of rumen contents both contribute to the effect of rumen fill on feed intake. Many researchers (Stokes et al. 1988; Baumont et al. 1990; Johnson and Combs 1991; Chilibroste et al. 1997; Akingbade and Hovell 1998) have found the

reduction of voluntary feed intake associated with addition of inert bulk or digesta. The rumen fill model of Van der Aar et al. (1983) indicated that volume of rumen fill explained less of the variation in feed intake than the weight of rumen fill. Selected research on relationship between fill and intake is presented below.

Schettini et al. (1999) altered mass and volume of rumen contents of steers. The addition of tennis balls to the rumen of steers reduced DM intake, and increasing the number of balls and specific gravity of the balls resulted in a further reduction of intake.

In a study by Dado and Allen (1995), Holstein cows in early lactation were fed diets containing with either 25% neutral detergent fibre (NDF) or 35% NDF. Inert bulk equal to 25% of pretrial rumen volume for each cow was inserted into the rumen. The researchers found that the addition of inert bulk reduced feed intake of cows fed the 35% NDF diet, but had no effect on the intake of cows fed the 25% NDF diet.

Johnson and Combs (1992) measured a reduction in feed intake upon addition of bladders to the rumen in mid-lactation when cows were fed a diet with a forage-to-concentrate ratio of 53:47. In a second trial they found the same result in mid- and early-lactation cows with diets containing forage to concentrate rations of 74:26 (33.2% NDF) and 50:50 (27.3% NDF).

Type of feed has an impact on fill. Rumen distension was a primary factor in limiting intake of hay, whereas physiological mechanisms were implicated in animals fed silage (Thiago 1988). Aitchison et al. (1986) found that sheep fed late-cut grass hay had low voluntary intake, but rumen pool sizes of DM, organic matter, and fibre were higher than clover hay. Gasa et al. (1991) concluded that intake of late-cut silage could have been limited by rumen fill, but not the intake of early-cut silage. Thiago et al. (1992) found that weight of rumen organic matter was higher in steers fed hay

compared to steers fed silage. Animals fed concentrates have lower rumen fill than animals on forage diets (Dulphy et al. 1996), and type of concentrate has been found to alter rumen fill (Carey et al. 1993).

Forage intake restriction has also been found to increase rumen fill in sheep fed wheatgrass hay (Tatman et al. 1991). This is supported by Chilibroste et al. (1999) who found that the DM rumen pools after grazing were higher in cows after 16.5 hours of starvation than in cows after 2.5 hours of starvation.

Although it is commonly accepted that rumen fill imposes a limitation on voluntary intake and thus determines voluntary intake with forage-based diets, there is not unanimous agreement on this concept. In the critical review of intake, Ketelaars and Tolkamp (1992) state that there is no conclusive role for rumen fill imposing restriction on intake. Rather, they suggest an important role for metabolic factors such as changes of basal metabolism and efficiency of utilization of metabolizable energy. Studies illustrate the ability of ruminants to increase roughage intake by speeding up passage of digesta or increasing rumen volume during periods of cold stress or lactation (Johnson and Combs 1991), which suggests that the extent of rumen fill is under the control of the animal and can be modified. Thus although gut fill may impose a limit on voluntary intake, this limit is not absolute.

2.2.1.2 Clearance of Undigested Particles

Ingested feed particles are removed from the digestive tract by digestion or passage. If rumen fill has an important effect on voluntary intake, the rate of clearance of undigested particles from the rumen must be an important factor in intake. In this regard, Guthrie and Wagner (1988) found rate of particulate passage and intake were highly correlated ($R = 0.98$). In other studies, however, these parameters are not as

highly correlated. This is because the daily clearance of particles from the rumino-reticulum is a function of both the amount of material in the rumino-reticulum (fill) and the rate of passage (% passing per h), and it is common for gut fill to increase when intake increases (Johnson and Combs 1991). Thus there can be an increased clearance of undigested particles from the rumen even though passage rates (expressed as percentage of particles in the rumino-reticulum passing per h) are similar.

Kovacs et al. (1998) fed steers at 1.0, 1.5, or 2.0 times the estimated maintenance energy requirements. With increasing intake the amount of fibre in rumen bailable liquids decreased, and there was more fibre in fecal particles at the high level of intake. The authors concluded that rates of passage of rumen fluid and particles increased linearly with increasing intake. Thornton and Minson (1973), Mudgal et al. (1982), Firkins et al. (1987), Robinson et al. (1987), Thiago (1988), Kreikemeier et al. (1990), Colucci et al. (1990), and Bosch et al. (1992) all had similar results.

Particle size and density are the primary feed factors associated with rate of passage of particles from the rumino-reticulum. Non-feed factors influencing clearance of particles from the rumino-reticulum include level of intake and diet composition.

Particles of 5 cm may exit the rumen, but most are less than 1 mm (Welch 1986). Kennedy (1995) found a curvilinear relationship between particulate passage rate from the rumen and particle size. Disappearance rate of particles between 0.071 and 1.25 mm was the rate-limiting step in control of rumen fill in the study of Bosch and Bruining (1995). Kaske et al. (1992) found that three to ten times more 1 mm particles were excreted than 10 and 20 mm particles.

Specific gravity of particles has also been shown to influence particulate passage rate. Particles with a specific gravity between 1.17 and 1.42 pass most rapidly.

Materials with a specific gravity of less than 1.0 are ruminated slowly, and therefore pass slowly. Conversely, increasing specific gravity above the optimal range results in a slower rate of passage and can decrease rumination (Welch 1986). These results are supported by research of Murphy et al. (1989) and Kaske et al. (1992). Hydration of forages in the rumen increases the functional specific gravity, making more feed particles available for passage. As a result high moisture forage have a higher particle clearance rate than low moisture forage (Welch 1986; Pasha et al. 1994).

The influence of rumen inert bulk on particle clearance rate from the rumen was studied by Schettini et al. (1999). Mass and volume of rumen contents of steers fed a low-quality forage diet were altered using varying numbers (50 or 100) and weights (specific gravity 1.1 or 1.3) of filled tennis balls. Increasing the number of balls in the rumen resulted in an increase in particle clearance rate. Increasing the specific gravity of the balls had no effect on rate of passage, but mean fecal particle size increased with heavier tennis balls. This supports earlier research on the effect of adding inert bulk to the rumen done by Okine et al. (1989), Johnson and Combs (1991), and Dado and Allen (1995).

Supplementation has been shown to increase particulate passage rate of forages (Guthrie and Wagner 1988; Sunvold et al. 1991). Concentrate level has more influence on clearance of low-quality forage than the clearance of grain, or high quality forage. Particle clearance rate for straw and hay remained unchanged when concentrate increased from 30 to 60%, but decreased by 28% and 13% for straw and alfalfa hay respectively as the level of concentrate increased to 90% (Poore et al. 1990).

2.2.1.3 Palatability

Palatability is defined as the pleasing or satisfying aspect of a feed (Van Soest 1994). It is the summation of taste, olfactory and textural characteristics of a feedstuff that determines its acceptance (Cheeke 1991).

Low palatability of poor-quality forages can result in reduced voluntary feed intake, especially when animals are given a choice. Weston and Davis (1986) found that intake of chopped straw decreased by 8% when a supplement of lucerne hay was offered as a separate meal once daily to sheep in comparison to when a mixture of lucerne hay and straw was provided. Gherardi et al. (1991) concluded that palatability had little effect on voluntary intake of forage when no choice is given, but when offered a choice between forages, animals consumed significantly more of the more palatable forage. Therefore, in feeding systems where choice is not offered palatability would be considered of little importance in influencing the consumption of the forage.

2.2.1.4 Physiological Factors

Physiological factors can have an impact on feed intake. Breed, gender, body fat, lactation, and pregnancy all influence feed intake. Lemm (2000) has reviewed the effects of breed, gender, body fat and lactation on voluntary feed intake in detail. A review by Allison (1985) discusses the effect of pregnancy on the intake of forages. This topic will not be discussed further here.

2.2.2 Diet and Feed Characteristics Affecting Intake and Digestion

2.2.2.1 Forage Quality

Forage quality is the totality of factors that influence the nutritive value of forages. The major factors relating to forage quality are digestibility, feed consumption, and the provision of nutrients (Cheeke 1991). Forage quality is affected by forage

maturity, environment and genotype. As digestibility increases so does the amount of available nutrients, and this can result in increased turnover in the rumen and increased intake.

Research by Shaver et al. (1988) studied the effect of forage maturity and genotype on digestion and digesta passage in dairy cows. Cows were fed lucerne hay harvested at pre-bloom, mid-bloom or full-bloom, or mature brome grass hay. Dry matter intake was lower for the brome grass than any of the lucerne hays, and weight of DM in the rumen was higher for the brome grass hay, mid-bloom and full-bloom lucerne hays than for the pre-bloom lucerne hay. Rumen retention of the digesta marker ytterbium (Yb) was longer for the brome grass hay than the pre-bloom lucerne hay, and fractional rates of in situ digestion of NDF were slower for full-bloom lucerne and brome grass than pre-bloom lucerne hay. It was concluded that feed intake was limited by gut fill, which was dependent on forage quality, and DM fill in the rumen was related to rates of ruminal digestion and passage.

Gasa et al. (1991) found that daily mean weights of wet digesta, liquid, NDF, and indigestible NDF in the rumen were greater with late-cut than early-cut silage.

Earlier research by Van der Aar et al. (1983) found that the intake of forages had a high correlation with NDF content of the forages, and that the cell wall structure and its digestion are associated. The fermentability of NDF increases as forage quality increases, and DM intake increases linearly as fibre fermentability increases (Robinson and McQueen 1997). This is supported by an analysis of 13 sets of forage comparisons reported in the literature by Oba and Allen (1999). They found that enhanced NDF digestibility of forage significantly increased DM intake; overall a one-unit increase in NDF digestibility in vitro or in situ was associated with a 0.17 kg increase in DM intake.

Diet composition also influences passage rate. Particulate passage rate decreases with increasing forage maturity (Park et al. 1994; Pasha et al. 1994; Bosch and Bruining 1995). As cell wall concentration increases, digesta retention time increases (Bosch et al. 1991). Stem fractions had a longer mean retention time than leaf fractions in hays fed to sheep (Cherney 1990). Thornton and Minson (1973) concluded that the intake of digestible organic matter was closely correlated with retention time of organic matter. This is supported by the research of Robles et al. (1981), where they fed sheep orchardgrass hay with varying cell wall concentrations (60 to 78%). They found that as digestible intake decreased, the sheep attempted to adapt by increasing ruminal ingesta volume and decreasing rate of passage.

2.2.2.2 Physical and Chemical Processing of Feed

There has been much research on the impact of physical and chemical processing of low-quality forages on voluntary feed intake. Grinding and pelleting result in an increase in feed intake as a result of increased particle clearance rate (Van Soest 1994). In a study by Uden (1988) pelleting lowered DM and NDF digestibility, decreased rumen fermentation rate, increased unavailable NDF fraction, and total solids retention decreased from 73 to 54 hours.

Various methods of chemical processing of low-quality forage have been investigated. Refer to Lemm (2000) for a more detailed review of physical and chemical processing of forages.

2.2.2.3 Supplementation of Nitrogen and Energy

Adequate nitrogen and limited amounts of readily available carbohydrates are required for optimum fibre digestion by rumen microorganisms (Nicholson 1984). Poor-

quality forages contain low quantities of both nitrogen and readily available carbohydrates (Theander and Åman 1984) and, therefore supplementation is required for efficient utilization of low-quality forages by ruminants.

2.2.2.3.1 Nitrogen Supplementation

Additional nitrogen can be supplied through either non-protein nitrogen (NPN) sources, such as urea, or through pre-formed proteins.

The supplementation of straw with urea was shown to increase intake of straw in research by Fishwick et al. (1974), Innes and Kay (1978), Cloete and Kritzinger (1984), and Mbtaya et al. (1985). In contrast, the addition of urea did not increase voluntary intake of barley straw in studies by Kay et al. (1968) and Alawa et al. (1988). The addition of 3% urea to a diet of oat straw supplemented with beet pulp did not increase the apparent digestibility of DM, but it tended to increase digestibility of crude protein (Kay et al. 1968; Fishwick et al. 1974; Cloete and Kritzinger 1984). In other studies, urea supplementation has been shown to increase organic matter and NDF digestibility (Punia et al. 1988; Djajanegara and Doyle 1989), where Brown et al. (1986) found that there was no difference in organic matter NDF, and ADF digestibility between untreated straw and that supplemented with urea.

The variability in the effect of urea supplementation made be due to the fact that low quality forages limit the use of NPN because of the high level of mature cell wall carbohydrates, which are slowly degraded. As a result, ammonia from NPN sources peaks before it is required by fermentation and is subsequently lost from the rumen and excreted as urea in the urine. Synchronization of the addition of NPN with readily available carbohydrates can improve the utilization of the NPN source (Van Soest 1994).

Supplementation of straw diets with pre-formed proteins has also been studied. Guthrie and Wagner (1988) studied the effect of protein supplements on intake and digestibility of prairie hay (4% protein) by beef steers. Researchers found that the high rate of protein supplementation (0.67kg of 34% protein) increased intake and digestibility of DM, organic matter, crude protein, ADF, and cellulose compared to the low rate of protein supplementation (0.36kg of 32% crude protein). Intake and digestibility was higher with the low rate of protein when compared to the control. In a second experiment, researchers found that added soybean meal resulted in a quadratic increase in forage intake, and digestibility.

Similar results were found by earlier research of Church and Santos (1981). It was found that voluntary intake of wheat straw was increased by the addition of 1 g soybean meal/$kg^{0.75}$ daily or more. The apparent digestibility of wheat straw alone was negative, but was increased by the addition of 1 g soybean meal /$kg^{0.75}$ or more and reached a plateau at 3 or 4 g soybean meal/$kg^{0.75}$. Improvement of intake and digestibility of straw when supplemented by pre-formed proteins was also found by Abidin and Kempton (1981), Capper et al. (1989), Silva et al. (1989), Ortigues et al. (1990), Zorilla-Rioset al. (1991), Beck et al. (1992), Guedes and Dias da Silva (1994), Warly et al. (1994), Fike et al. (1995), and Mawuenyegah et al. (1997).

2.2.2.3.2 Energy Supplementation

According to Van Soest (1994) the utilization of NPN sources may be limited if a sufficient quantity of readily available carbohydrates is not present in the rumen. Results of Zorilla-Rios et al. (1989) confirm that straw diets need to be supplemented with a suitable source of energy to increase utilization of added N.

Intake of barley straw was increased from 48 g/kg$^{0.75}$ to 58 g/kg$^{0.75}$ when the straw was supplemented with 1 kg of barley grain with added urea, but this fell to 52 g/kg$^{0.75}$ when no urea was offered in the study of Innes and Kay (1978). Capper et al. (1989) found that supplementation of barley straw with barley grain increased organic matter intake for 2-row barley straw varieties and not for the 6-row varieties. Provision of a starch concentrate up to 31% of the whole diet increased voluntary feed intake and the microbial protein per unit digestible organic matter intake (Gomes et al. 1994; Castrillo et al. 1995).

Guthrie and Wagner (1988) demonstrated that a grain supplement (1.41 kg of 13% crude protein) had similar effects as a low rate of protein supplementation (0.36 kg of 32% crude protein) in terms of increased forage intake and digestibility of DM, organic matter, crude protein, ADF, and cellulose. Silva et al. (1989) demonstrated that sugar-beet pulp increased DM and organic matter digestibility of urea supplemented straw. Sultan and Loerch (1992) confirmed these results. Rumen and total tract DM and organic matter digestion was 41% and 33% greater in high-energy diets than in low-energy diets, and N digestibility was higher in the high-energy diets compared to the low-energy diets. Retention of N was also greater with the high-energy diets than with the low-energy diets.

Beck et al. (1992) found that sorghum grain with soybean meal decreased NDF digestibility, and that a high level of sorghum grain (2.72 kg/day) had a large decrease compared to sorghum grain plus soybean meal. Researchers also found that insoluble ADF fill and passage rate was not affected, but liquid dilution rate was slightly increased with energy supplementation and decreased with protein supplementation. This is supported by Mawuenyegah et al. (1997) where ammoniated straw was supplemented

with soybean meal and molasses meal resulted in reduced DM and fibre digestion, but increased intake and microbial synthesis.

2.2.3 Concluding Remarks

As evidenced by the extensive amount of research on the feed intake of ruminants we can surmise that there is no single control of intake. Instead intake is controlled by a complex system of physiological and metabolic factors. We can influence intake via the type of feed, chemical and physical processing and supplementation of energy and/or protein, but the main control remains with the animal as indicated by its ability to adjust feed intake beyond apparent physical limitations in adverse conditions.

2.3 Effect of Frequency of Supplementation on Intake, Ruminal Conditions, and Degradability of Straw

2.3.1 Dry Matter Intake

2.3.1.1 Supplementation More than Once Daily

The objective of this study was to look at the impact of supplementation of straw diets less than one time per day. For more information regarding the effect of supplementing more than once daily refer to Lemm (2000).

2.3.1.2 Supplementation Less than Once Daily

There is limited work done on the supplementation of forage diets less than once daily, and the research that has been done has found variable results. Feed intake, NDF intake and total intake in winter-grazing pregnant cows were not affected by form of lucerne (pellet or hay) or frequency (daily or alternate days) of feeding lucerne (Brandyberry et al. 1992), and hay organic matter intake was not affected by

supplementation frequency (Chase and Hibberd 1989). In contrast, Garza et al. (1992) found that intake of prairie hay was increased by urea infusions, and that the best intake response was when urea was infused once daily or on alternate days compared to twice per day.

Collins and Pritchard (1992) found that DM intake was variable in response to protein source and frequency. Recent research by Huston et al. (1999) found that supplement and forage intake was more variable within groups with once daily supplementation compared to when a supplement was fed less frequently. It was also concluded that supplementation once per week was as effective as daily supplementation in terms of reducing loss of live bodyweight and body condition score.

McIlvain and Shoop (1962) found no significant difference in the live weight gain of steers fed cottonseed cake every third day compared to every day. Research by Pate and Crockett (1971) concluded that steers fed a high protein supplement every third day gained more compared to those steers fed the high protein supplement every day. In contrast, Beaty et al. (1994) found that decreasing supplementation frequency increased winter weight loss.

2.3.2 Effect of Supplementation Frequency on Rumen Environment and Metabolism

2.3.2.1 Fermentation of Feeds

Fermentation of feeds in the rumen is dependent on external factors such as amount and composition of the diet. Optimal fermentation is achieved only when nutritional requirements of the microorganisms are met, and the rumen environment is adequate. If requirements of the rumen microorganisms cannot be met, either intake or digestibility will decrease as a result of a slower rate of digestion (Van Soest 1994).

Abrupt changes in the diet may also impact rumen fermentation by changing the environment of the rumen. As a result, the changing rumen environment favors a different composition of rumen microorganisms which then causes a shift in the fermentation balance (Van Soest 1994).

2.3.2.2 Ruminal pH

In general, a pH of less than 6 has been implicated in the reduction of cellulolytic activity, although substrate inhibition has been proven at higher pH (Van Soest 1994). Normal rumen pH hovers in the range of 6 to 7. The absorption of fermentation acids, and the neutralization of acidic byproducts of fermentation by salivary buffers helps maintain the rumen pH in this range.

In the study by Chase and Hibberd (1989) the mean rumen pH of heifers given 4.1 kg of a corn supplement on alternate days was lowest on days when the supplement was fed (6.30) and highest (6.63) on days when no supplement was fed. The ruminal pH of the heifers fed the high level of maize on alternate days remained below 6.25 for at least 12 h after supplementation. In addition they found no difference in mean rumen pH on days when animals were provided 1.4 kg (Low) of supplement daily or 2.8 kg (High) of supplement on alternate days (6.40 vs. 6.41). On days when no supplement was fed in the alternate day treatments, the rumen pH was higher compared to animals fed the Low (6.41 vs. 6.51) and High (6.46 vs. 6.63) supplemental treatments daily.

Similar results were found by Beaty et al. (1994). They also found that ruminal pH of steers supplemented 3 times per week was lower than that of the steers supplemented 7 times per week on days that both groups received supplement, but the pH never fell below pH 6 (Fig. 2.1). In addition, the rumen pH of the steers

supplemented 3 times per week remained higher throughout the day when no supplement was fed than the 7 times supplemented steers.

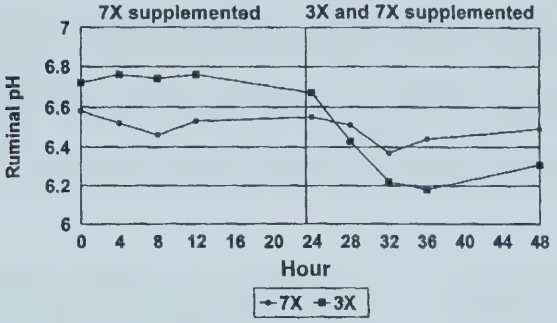

Figure 2.1. Influence of frequency of supplementation of beef steers fed unrestricted amounts of wheat straw on ruminal pH. Standard error of the mean = .18 (Exp. 1). (from Beaty et al. 1994)

Collins and Pritchard (1992) found no difference in mean rumen pH between animals fed a corn-stalk based diet supplemented with soybean meal (6.45 vs. 6.43) or corn gluten meal (6.38 vs. 6.44) every 24 or 48 h respectively. There was a significant diet x hour interaction, however it was of limited importance because all the pH values were well within the 6.3 to 6.8 range normally associated with high roughage diets.

2.3.2.3 Rumen Ammonia

The NRC (1996) states that bacterial crude protein can supply from 50% to essentially all of the metabolizable protein required by beef cattle. Many microorganisms that ferment carbohydrates in the rumen require ammonia. In general the optimal level of ammonia in the rumen is 10 mg dL^{-1} according to Van Soest (1994) and 2 to 5 mg dL^{-1} according to Satter and Slyter (1974). Availability of carbohydrate in the rumen promotes utilization of ammonia, and the rate of carbohydrate fermentation

can determine capacity for ammonia uptake and protein synthesis by bacteria (Van Soest 1994).

Chase and Hibberd (1989) found that rumen ammonia levels peaked at 3 hours after feeding on days with or without maize supplementation. In heifers fed corn supplements on alternate days ruminal ammonia levels were never above 1.0 mg dL^{-1} on the day that no supplement was fed, and remained as such until the subsequent feeding.

In the research of Beaty et al. (1994) rumen ammonia levels peaked at 4 hours post-supplementation in steers supplemented daily (all protein levels), and in steers fed the 12 and 20% crude protein supplements three times per week (Fig. 2.2). Levels of rumen ammonia declined steadily throughout the day in 3 times steers on days when they were not supplemented. Rumen ammonia levels of steers supplemented three times per week with 30% and 39% crude protein supplements peaked later and at higher concentrations than in the steers fed 30% and 39% CP supplements daily.

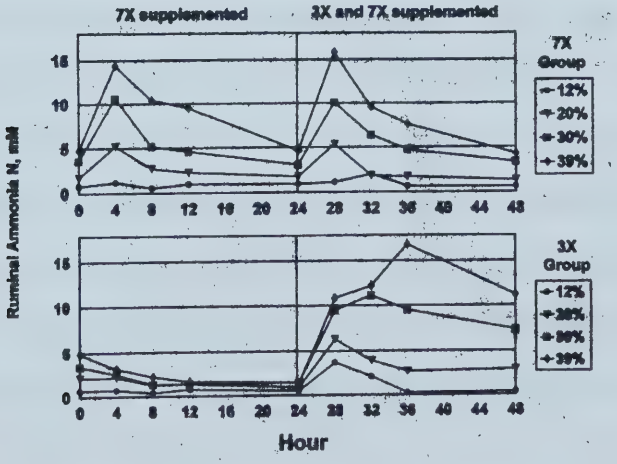

Figure 2.2. Influence of protein concentration in supplements and frequency of supplementation of beef steers fed unrestricted amounts of wheat straw on ruminal NH3 N concentrations. Standard error of the mean = 1.74 (Exp. 1). (from Beaty et al. 1994)

Collins and Pritchard (1992) found that mean ruminal ammonia N levels were highest (10.4 mg dL^{-1}) when soybean meal was provided every 48 h to sheep fed a corn stalk diet. There was no difference between feeding soybean meal every 24- or 48-h, or between corn gluten meal fed every 24- or 48-h. Feeding corn gluten meal every 48-h resulted in similar ruminal ammonia N levels as feeding soybean meal every 24-h. Ruminal ammonia N levels never went below 2 mg dL^{-1} in any of the treatments and sampling times.

2.3.2.4 Rumen Volatile Fatty Acid Concentrations and Proportions

Volatile fatty acids (VFA) are the end product of microbial metabolism and are the main source of metabolizable energy for the ruminant. They include acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids.

Collins and Pritchard (1992) found that diet did not influence total VFA concentration in sheep fed a corn stalk diet supplemented with either soybean meal or corn gluten meal. Total VFA patterns were similar between soybean meal and corn gluten meal fed at 48-h intervals. Peak total VFA concentrations were less responsive to corn gluten meal than soybean meal when fed every 24-h. Molar proportions of acetate:propionate:butyrate were 74:18:7, 74:18:7, 74:18:8, and 74:18:8 for soybean meal fed every 24- or 48-h and corn gluten meal fed every 24- or 48-h respectively, indicating similar fermentation pathways. However, Collins and Pritchard (1992) did find that there were treatment effects on individual VFA concentrations. Isobutyrate concentrations were lower when corn gluten meal was provided every 24 h than in other treatments.

Chase and Hibberd (1989) found that alternate day supplementation tended to lower total VFA levels, but molar proportions of propionate tended to increase with

alternate day supplementation. Thus, acetate:propionate ratios tended to decrease when supplements were provided on alternate days. Chase and Hibberd (1989) concluded that because cows supplemented on alternate days were fed twice the daily amount of corn at feeding might exaggerate the typical concentrate effect on VFA proportions. They suggested this may imply that providing corn supplements every other day might utilize their diet less efficiently than cows supplemented every other day.

2.3.3 Digestibility

Coleman and Wyatt (1982) found that DM digestibility (50.6, 49.9, 51.7%) and crude protein digestibility (54.9, 53.4, 55.7%) was not affected by providing a cottonseed meal supplement daily, every other day, or every fourth day respectively, to steers on native grass hay (7.9% CP; 45.5% ADF). In another experiment by Coleman and Wyatt (1982), steers fed native grass hay (3.3% crude protein; 53.9% ADF) were supplemented with small grains forage as the protein source. Again, the supplement was provided daily, every other day, or every fourth day. Dry matter digestibility was significantly lower for those animals supplemented every fourth day than for those fed every other day or daily (40.6 vs. 44.6 or 45.8% respectively). The same result was obtained for crude protein digestibility (41.7 vs. 48.6 or 49.0% respectively).

The research of Chase and Hibberd (1989) also found that total digestibility, and hay organic matter digestibility, and apparent crude protein digestibility were not reduced by alternate-day feeding in heifers fed grass hay and provided with a maize supplement.

Dry matter disappearance (%) and nitrogen digestibility was not affected by frequency (daily or alternate days) of protein supplementation (soybean meal or corn gluten meal) of corn stalk diets (Collins and Pritchard 1992). Dry matter disappearance

for soybean meal was 62.1 and 57.4% and for corn gluten meal it was 57.6 and 60.6% for the 24 h and 48 h feeding frequencies respectively. Nitrogen digestibility was 61.8 and 59% for soybean meal and 59.5 and 62.06% for corn gluten meal fed every 24 or 48 h, respectively. Brandyberry et al. (1992) found similar results.

In contrast, Beaty et al. (1994) found that decreasing the supplementation frequency from once daily to three times per week increased DM and NDF digestion (49.6 vs. 54.2%; 51.1 vs. 54.4% respectively).

2.3.4 Passage

Coleman and Wyatt (1982) found that frequency (daily, alternate days, or every fourth day) of supplementation of steers fed native grass hay (3.3% CP) with small grains forage had no effect on passage rate (3.53, 3.24, 2.92% h^{-1}). However, there was a trend towards decreasing passage rate with decreasing frequency of supplementation.

Chase and Hibberd (1989) provided a more detailed study of changing ruminal kinetics in response to different supplementation frequencies. Heifers were fed a corn stalk diet and supplemented with a low level of maize (1.4 kg d^{-1}) or a high level of maize (2.0 kg d^{-1}) daily, or at twice the daily amount on alternate days (2.8 or 4.1 kg). Particulate passage rate (3.7, 3.9, 3.5, 3.4% h^{-1}) and liquid dilution rate (7.8, 7.8, 8.4, 7.7% h^{-1}) did not differ for any of the treatments, low-daily, low-alternate days, high-daily, or high-alternate days respectively.

2.3.5 Concluding Remarks

Researchers to date have demonstrated that alternate day feeding has not had a significant impact on the rumen environment, which would ultimately shift the microbial

population and fermentation dynamics. No significant change in the digestibility of various feed components in animals fed supplements on alternate days lends credence to the previous statement. The question is how far can we go? Some research has studied the effect of feeding even less frequently than alternate days. Early results seem to be similar to those studies done on feeding on alternate days, but there is not enough research to truly assess this issue. Current research has demonstrated that providing supplements less than once daily has great potential to be a viable option for cattle producers.

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CHAPTER 3

Effects of Timing of Concentrate Feeding on Dry Matter Intake, Ruminal Degradability of Straw and In Vivo Digestibility

3.1 Introduction

Winter feeding costs are the single greatest cost of production for most cow-calf producers, averaging approximately 33% of these costs in 1998 (AAFRD 1999). One way of reducing feed costs is by feeding low quality forages, such as cereal straw, to dry and pregnant cows. Cereal straw alone cannot meet the maintenance requirements of the beef cow. As a result, such diets must be supplemented with concentrates or high quality forage to meet the nutritional requirements of the cow.

Some producers in Alberta and the U.S.A. are now providing concentrates at a frequency of less than once daily in order to reduce labour and equipment costs. Several studies have shown that performance was not significantly impacted by reducing feeding frequency of protein supplements to less than once daily in grazing animals (McIlvain and Shoop 1962; Pate and Crockett 1971; Huston et al. 1999). It has been shown that intake and digestibility have not been influenced by supplementation frequency (Coleman and Wyatt 1982; Chase and Hibberd 1989; Hunt et al. 1989; Collins and Pritchard 1992). There is, however, only one study (Beaty et al. 1994) in which a reduced frequency of feeding of concentrates to cattle consuming cereal straw-based diets has been examined.

The objectives of this study are to investigate the influence of providing a low or high protein supplement either daily, on alternate days or every third day on DM intake, ruminal disappearance of straw, and in vivo digestibility. Our hypothesis was that protein intake would affect these parameters but frequency of feeding concentrates would have no effect.

3.2 Materials and Methods

3.2.1 Animals and Feed

Five crossbred steers (465 ± 30 kg) were used in a 5 x 5 Latin square design (every animal was fed every supplemental treatment) experiment conducted at the Laird McElroy Environmental and Metabolic Center, University of Alberta, Edmonton Research Station, Edmonton, Alberta, Canada. At least 90 days before the experiment, the steers were fitted with 10-cm i.d. soft ruminal cannula (Bar Diamond, Parma, ID), using aseptic technique and local anaesthetic (2% lidocaine). Antibiotic therapy after surgery was procaine Penicillin G (300,000 IU ml^{-1}: 2 ml 100 kg^{-1} body weight intramuscularly). Steers were maintained indoors in individual 3 m x 3 m pens with continuous lighting, and an average ambient temperature of 17°C throughout the experiment. All animals were cared for in accordance with the guidelines of the Canadian Council of Animal Care (1993), and under the advice of the Faculty of Agriculture, Forestry, and Home Economics Animal Policy and Welfare Committee.

All steers were offered barley straw (Lacombe, six-row) ad libitum along with five different supplemental concentrate treatments. Concentrate treatments were as follows: 1) low protein concentrate fed daily (Low-1), 2) low protein concentrate fed every second day at two times the daily rate (Low-2), 3) high protein concentrate fed daily (High-1), 4) high protein concentrate fed every second day at two times the daily rate (High-2), 5) high protein concentrate fed every third day at three times the daily rate (High-3). Rate of concentrate supplementation was calculated as 30% of the total as-fed intake of the previous week. Treatments are summarized in Table 3.1.

Ingredients used in the concentrates and their composition are summarized in Table 3.2, as is information on the composition of the straw. Steers were also provided

with free access to fresh water and trace mineralized salt blocks. The straw was chopped in a tub grinder (New Holland Model 390, Sperry, New Holland, PA) to approximately 6 cm in length. The straw contained 5.3% crude protein (CP), 76.9% neutral detergent fibre (NDF), and 52.6% acid detergent fibre (ADF). The average crude protein content of six-row barley straw in Alberta in 1984-1994 was 5.4% (AAFRD 1997), suggesting that the barley straw used in this experiment was of average quality.

Straw was offered ad libitum in such amounts as to maintain approximately a 10% weighback. Concentrates were offered at 0900h on the days that they were provided. All of the steers were allowed to consume the concentrate before the straw was offered (normally within 0.5 hour). On those days when animals were not fed concentrates they were offered the straw at 0900 h. On some occasions, not all of the concentrate was consumed in the time allotted. In such cases, the straw would be added on top of the concentrate.

Animals were weighed before each period. Each period was designed to be 31 days in length, although due to practicalities of scheduling facilities and equipment actual periods ranged from 26 to 35 days. There was a 14-day adaptation period prior to sampling. Voluntary feed intake measurements were obtained during days 15 to 20. During this time nylon bags were also incubated in the rumen, fecal 'grab' samples were obtained for calculation of digestibility, and urine samples were collected (data are not reported for urine composition or volume). Rumen samples were taken between days 23 and 31 for determination of metabolite concentrations (Chapter 4) and markers were administered for determination of ruminal fluid dilution rates and particle retention times (not reported). Indirect calorimetry measurements (not reported) were obtained between days 21 and 31.

Orts were removed and weighed prior to feeding the next day. Samples of straw, concentrates, and orts were taken daily for a 6-day period and composited separately for the period. The composite samples were used for analysis. Feed, orts, and fecal samples were dried in a forced air oven (Despatch V Series, Despatch Industries, Inc. Minnesota) at 60°C until a constant weight. Dried samples were ground to pass a 1mm screen prior to analysis.

3.2.2 In Situ Degradability in Nylon Bags

Measurements of ruminal disappearance of straw after 24, 48 and 72 h in situ incubations took place during days 15 to 20. Straw samples were ground (Thomas Mill Model 4, Philadelphia) through a 2 mm screen and 2 to 3 g (20 to 30 mg cm^{-2}) were placed in 5 cm x 10 cm polyester mesh bags with a pore size of 50μm (ANKOM Technology Corporation, Fairport, NY). After filling the bags were sealed with an elastic band. Three bags were prepared for each incubation time for each of the five steers. There were thus three replications for each sampling time for steers fed concentrate daily but only one replication for each sampling time for treatments in which concentrates were not fed daily. Samples were introduced in reverse sequence; the 72-h bags were placed in the rumen first, then the 48-h bags, followed by the 24-h bags. After removal from the rumen, nylon bags were frozen at -20°C until they could be washed simultaneously in a conventional washing machine. The bags were dried at 60°C to constant weight and weighed to determine percent dry matter disappearance.

3.2.3 In Vivo Apparent Digestibility

Apparent digestibilities of dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), protein and gross energy were determined by the digesta marker

technique, which assumes that the marker passes through the digestive tract without being absorbed. Chromium-mordanted fibre (Cr-fibre) made from straw and cobalt-EDTA was prepared according to Uden et al. (1980). Animals were dosed intraruminally with 10 g Cr-fibre (3.1% Cr), and 10 g Co-EDTA (12.7% Co) every six hours starting 72 hours prior to fecal sample collection. Mean doses actually delivered during the experiment were 1.15 and 4.68 g daily of chromium and cobalt, respectively. Fecal samples were obtained either after voluntary defecation or by rectal grab sample six times daily over the 6-day period. A 200 g sample was taken at each sample time and composited on a daily basis.

Fecal DM produced daily (g) was calculated from the mean concentration of marker in feces as:

$$\frac{\text{Daily marker dose (mg)}}{\text{fecal marker concentration (mg/g)}}$$

Intakes were averaged over the 6 day period for both straw and concentrates, then apparent digestibilities (%) were calculated as:

$$\frac{((\text{DM intake} * \text{nutrient concentration}) - (\text{fecal DM} * \text{nutrient concentration})) * 100}{(\text{DM intake} * \text{nutrient concentration})}$$

3.2.4 Chemical analysis

Crude protein was determined on approximately 100-mg samples using a nitrogen analyzer (LECO Model FP-428, St. Joseph, MI).

Neutral detergent fiber (NDF) was determined according to the procedure of Van Soest et al. (1991) without amylase or sodium sulfite. The Association of Official Analytical Chemists (1997) procedure #973.18 was used for the determination of ADF. Fibre analysis was conducted using an ANKOM^{200} Fiber Analyzer (ANKOM Technology

Corporation, Fairport, NY) with filter bags. Lignin was measured with the 72% sulfuric acid procedure of Goering and Van Soest (1970).

Gross energy was measured with a Parr adiabatic bomb calorimeter (Parr Instrument Co., Inc. Moline, IL).

Chromium and cobalt contents in ruminal and fecal samples were determined by procedures outlined in Okine et al. (1989).

3.2.5 Statistical Analysis

Mean concentrate and straw intakes over the 6-day period for each animal were analyzed as a 5 x 5 Latin Square design using the GLM procedure of SAS (SAS Institute, Inc. 1988). Treatments (n = 5), periods (n = 5) and animals (n = 5) were the main sources of variation. One animal was not included in analyses of straw DM intake (High-1 diet) because of a brief illness during one period. Means were separated using the Student-Newman-Keul's test (SAS Institute, Inc. 1988).

Straw disappearance data were analyzed in a similar manner within each incubation time. Comparisons between both dietary protein and frequencies of feeding concentrates were obtained by using contrast statement in SAS (SAS Institute, Inc. 1988), with only the Low–1, Low-2, High-1, and High-2 treatments being considered in these comparisons. Mean ruminal disappearances for each dietary treatment were determined after calculating mean values for all samples incubated within each animal for each period. The repeated measures component of the GLM procedure of SAS (SAS Institute, Inc. 1988) was used to determine the overall effect of treatment on straw degradability with incubation time as the repeated measure. Finally, the effect of adding bags to the rumen on days when concentrate was fed versus days when concentrate

was not fed was determined within each dietary treatment and time, with treatments and animals as sources of variation.

In vivo apparent digestibility data were also analyzed as a 5 x 5 Latin Square designed experiment with protein and feeding frequency contrasts. The GLM procedure of SAS (SAS Institute, Inc. 1988) was also used to determine the effect of day of concentrate feeding on calculated apparent digestibility.

3.3 Results

3.3.1 Feed Intake

Mean daily DM intakes of straw and concentrate and total intake over the experimental period are outlined in Table 3.3.

Straw DM intake was 5.53, 5.56, 5.34, 5.42, 4.90 kg d^{-1} (P = 0.17) for Low-1, Low-2, High-1, High-2, and High-3 diets, respectively. Corresponding DM intakes as a percentage of bodyweight were 1.18, 1.18, 1.14, 1.17, and 1.04% (P = 0.20). Straw intake when the low protein supplements were fed (5.55 kg d^{-1}) was similar to straw intake when the high protein supplements were fed (5.39 kg d^{-1}). Mean daily straw intake when concentrate was fed daily was 5.45 kg in comparison with the intake of 5.49 kg in steers fed concentrate every second day (P = 0.99).

The amount of concentrate offered was calculated as 30% of feed intake the previous week, and was dependent on the amount of straw eaten. Intakes of concentrate DM were 2.45, 2.10, 2.60, 2.48, 2.21 kg d^{-1} (P = 0.03) for steers on the Low-1, Low-2, High-1, High-2 and High-3 treatments, respectively. Corresponding percentages of concentrate in the diet were 31, 27, 33, 31 and 31, 31%, which differed slightly from the targeted 30%. As a consequence, steers given the high protein concentrates either daily or every second day consumed a diet containing 10% more

concentrate (32 vs. 29%) in comparison with steers fed the low protein concentrate. Also, steers fed concentrate daily consumed a diet which contained 10% more concentrate than steers fed concentrate every second day (32 vs. 29%, respectively). These unplanned for and unexpected differences were unfortunate but would not be enough to affect the interpretation of our results. On average, concentrate intake was 0.5% of body weight (Table 3.3).

Total mean daily DM intake was 8.00, 7.66, 7.95, 7.90, and 7.11 kg d^{-1} for Low-1, Low-2, High-1, High-2, and High-3 respectively ($P = 0.05$; Table 3.3). Corresponding intakes as a percentage of body weight were 1.70, 1.63, 1.69, 1.70, and 1.51% ($P = 0.56$). Steers fed the low protein concentrate diets consumed 7.82 kg d^{-1} which was similar ($P = 0.52$) to the 7.92 kg d^{-1} of those fed the high protein concentrate diets. Similarly, intakes were similar ($P = 0.30$) in steers fed concentrate daily (7.97 kg d^{-1}) and those fed concentrate every second day (7.78 kg d^{-1}).

The effect on straw intake of whether or not concentrate was fed on a particular day is examined in Table 3.4. Straw intakes did not differ between the day on which concentrate was fed and the days on which concentrate was not fed for the Low-2, High-2 and High-3 treatments.

3.3.2 Ruminal Disappearance of Barley Straw

Ruminal disappearances of straw at 24, 48, 72 hours and the mean of these times are shown in Table 3.5. As expected, there was an effect ($P < 0.01$) of length of time incubated in the rumen on ruminal disappearance of straw.

Twenty-four hour straw disappearances were 37.0, 37.9, 42.1, 40.1, and 40.1% for Low-1, Low-2, High-1, High-2, and High-3, respectively ($P = 0.02$). Feeding the high protein concentrate compared to feeding the low protein concentrate increased ($P <$

0.01) 24 h disappearance of straw by 10% (from 37.5 to 41.1%) when concentrates were fed daily or every second day. Frequency of feeding protein supplement did not impact the 24h degradability of straw in the rumen (39.6% when concentrate was fed daily in comparison to 39.0% when concentrate was fed every second day).

Forty-eight hour straw disappearances were 50.1, 49.9, 51.5, 49.6, and 49.4% for Low-1, Low-2, High-1, High2, and High-3, respectively (P = 0.60). Protein level in the diet did not influence 48 h straw degradability when concentrates were fed daily and every second day, with degradabilities of 50.0 and 50.5% (P = 0.58) for the low and high protein diets, respectively. Similarly, frequency of feeding concentrates did not impact the 48h degradability of straw in the rumen (50.8% when concentrate was fed daily in comparison to 49.8% when concentrate was fed every second day).

Seventy-two hour straw disappearances were 55.0, 55.6, 56.0, 54.3, and 53.7% for Low-1, Low-2, High-1, High2, and High-3, respectively (P = 0.07). Protein level in the diet did not influence (P = 0.79) 72 h straw degradability when concentrate was fed daily and every second day, with degradabilities of 55.3 and 55.2% for the low and high protein diets, respectively. Similarly, frequency of feeding protein supplement did not impact (P = 0.32) the 72-h degradability of straw in the rumen (55.5% when concentrate was fed daily in comparison to 55.0% when concentrate was fed every second day).

Mean ruminal straw disappearances were 47.4, 47.8, 49.9, 48.0 and 47.8% for Low-1, Low-2, High-1, High-2, and High-3, respectively (P = 0.10). Protein level in the diet affected (P = 0.05) overall straw degradability when concentrate was fed daily and every second day, with degradabilities of 47.6 and 48.9% for the low and high protein diets, respectively. Frequency of feeding protein supplement, however, did not impact

(P = 0.27) the overall degradability of straw in the rumen (48.6% when concentrate was fed daily in comparison to 47.9% when concentrate was fed every second day).

The effect of day on which bags were placed in the rumen on straw disappearance is shown in Fig. 3.1 (also in Table A1 in the Appendices). There were no differences in straw disappearance when bags were placed in the rumen on days when concentrate was fed in comparison with placing bags in the rumen on days when concentrate was not fed.

3.3.3 In vivo digestibility

There were substantial differences between estimates of digestibility depending upon which marker was used; mean DM digestibilties across all treatments with lignin, cobalt-EDTA and chromium-fibre markers were 56, 64 and 70%, respectively (Table 3.6).

When lignin was used as a marker differences ($P < 0.1$) in digestibility due to treatment were observed for all items measured except NDF (Table 3.6). The differences were due to protein level in the concentrate; when concentrates were fed daily or every second day digestibilities with high protein concentrate were 5, 4, 8, 33 and 6% higher ($P < 0.11$) for DM, NDF, ADF, crude protein and energy, respectively, than with the low protein concentrate. Digestibility was not affected by feeding concentrate every second day rather than daily (Table 3.6). When cobalt-EDTA or chromium-fibre were used as markers, no differences in digestibility were detected due to treatment with the exception of crude protein digestibilities. The digestibilities of crude protein were 19 and 13% higher ($P < 0.05$) with cobalt-EDTA and chromium-fibre, respectively, in steers fed the high protein diet either once daily or every second day than in steers fed the low protein diet. There were also trends ($P < 0.1$) for energy

digestibility and digestible energy content of the high protein diets to be 3% higher than low protein diets when cobalt-EDTA was used as a marker.

Digestibility estimates were also made for days on which concentrates were fed and days when concentrates were not fed using mean daily fecal marker concentrations and the mean DM intake over the 6 d period in the calculation (Table 3.7). These values were compared with the treatments in which concentrates were fed daily. With lignin as the marker, the only differences detected between days were for crude protein digestibility for steers fed concentrate once every 3 days, where the calculated digestibility was 13% higher ($P = 0.03$) when concentrate had not been fed for 2 days than on the day of concentrate feeding (Table 3.7). A similar difference was seen when chromium was used as a marker (Table A3), but differences were not significant with cobalt (Table A2).

3.4 Discussion

3.4.1 Voluntary Consumption of Straw-based Diets

Voluntary consumption of straw averaged 1.18, 1.18, 1.14, 1.17, and 1.04% of body weight (Table 3.3) for animals fed the low protein concentrate daily (Low-1), the low protein concentrate every other day (Low-2), the high protein concentrate daily (High-1), the high protein concentrate every other day (High-2), and the high protein concentrate every third day (High-3). These straw intakes are similar to those of Zorrilla-Rios et al. (1991) who measured intakes of untreated straw as 1.08, 1.14, and 1.17% of body weight when animals were supplemented with 0, 150, or 500 g d^{-1} soybean meal, respectively. However, straw intakes were slightly lower than the intakes measured by Johnson (1972), Mathison et al. (1981), and Okine et al. (1993). Mathison et al. (1981) found mean daily straw intakes of 1.4% of body weight when feeding straw-

based diets supplemented with concentrates. Intake of straw in the study of Okine et al (1993) was 1.4% of body weight when a diet containing 93% straw was fed. Beaty et al (1994) measured a daily intake of straw of 1.4% when animals had supplemental concentrate supplied daily and 1.18% when animals were supplemented three times per week, the latter being close to our intakes.

In this study, protein supplementation did not increase voluntary intake of straw. This is supported by the research of Kay et al. (1968) and Alawa et al. (1988), which found that the addition of urea did not increase voluntary intake of barley straw. In contrast, others have found that urea supplementation had a positive effect on straw intake (Fishwick et al. 1974; Innes and Kay 1978; Cloete and Kritzinger 1984). Feeding preformed proteins has most consistently resulted in an increase in intake. Sunvold et al. (1991) found a linear increase in DM intake of bluestem-range forage when feeding 15, 20, and 25% crude protein supplements. Beaty et al. (1994) also found a linear increase in straw intake when supplements containing 12, 20, and 30% protein were fed, but measured a decrease in straw intake with a supplement containing 39% protein.

Neither straw intake nor total intake was affected when steers were supplemented less frequently than once daily, although straw intake was numerically 8% less when the high protein concentrate was provided every third day than when it was provided daily (Table 3.3). Supplementing straw-based diets less than once daily has been shown to have variable effect on intake. Intake of wheat straw fell from 1.42% for animals supplemented daily to 1.18% body weight for animals fed three times per week with a soybean meal/sorghum grain supplement in the study of Beaty et al. (1994). Coleman and Wyatt (1982) found no difference in DM intake of steers fed native range hay (8% crude protein) and supplemented daily, every other day, or every fourth day

with cottonseed meal. However, they did find that feeding a small grains forage supplement every fourth day resulted in decreased total intake compared to daily and every other day. Hunt et al. (1989) reported similar results in steers fed grass hay (6.6% crude protein) and provided with a cottonseed supplement every 12, 24, or 48 h. Chase and Hibberd (1989) did not measure a difference in intake between cows fed low-quality grass hay (5% crude protein) and supplemented either a low level (1.4 kg daily equivalent) or a high level (2.0 kg daily equivalent) of corn daily or on alternate days. Huston et al. (1999) found that supplement and forage intake was more variable within groups with once daily supplementation compared to when a supplement was fed less frequently. Collins and Pritchard (1992) concluded that DM intake was variable in response to protein source and frequency.

Performance of animals fed supplements less than once daily has also been variable. McIlvain and Shoop (1962), Brandyberry et al. (1992), and Huston et al. (1999) all concluded that there was no difference in performance of animals supplemented less frequently than those supplemented daily. Pate and Crockett (1971) found that steers fed a high protein supplement every third day gained more than those fed the same supplement daily. In contrast, Kartchner and Adams (1982) and Beaty et al. (1994) measured a decrease in animal performance when fed supplements less frequently than daily.

The observation that straw intake did not differ between days when concentrate was fed and days when concentrate was not fed (Table 3.4) is of interest. This suggests that the steers did not change straw intake in anticipation of changes in availability of concentrate. Moreover it is consistent with the concept that concentrate does not influence ruminal fill and hence intake.

3.4.2 In Situ Disappearance of Straw

The polyester bags contained 20 to 30 mg of sample per cm^2 of bag surface. This relatively high weight to surface area ratio was used by 16% of researchers in the summary of Vanzant et al. (1998). This is higher than the recommended value of 10 mg cm^{-2}, but according to Vanzant et al. (1998), the effect of weight per surface area is not great when slowly degrading forages such as straw are used. The use of a 2-mm screen for grinding and a 50-micrometer pore size are consistent with recommendations of Vanzant et al. (1998).

Frequency of provision of concentrate did not have an effect ($P > 0.05$) on 24, 48, or 72 h ruminal degradability of straw (Table 3.5). These results are consistent with those of Hunt et al. (1989) who found that in situ fibre degradability of grass hay was not affected by feeding cottonseed meal every 12, 24, or 48 hours.

There was a 10% increase ($P < 0.01$) in ruminal disappearance of straw after 24 h incubation when the high protein concentrates were fed in comparison to the low protein concentrates. Such a difference was not apparent at either 48 or 72 h but the mean disappearance was 3% higher ($P = 0.05$) when the high protein concentrate was fed over all incubation times. These results suggest that the rate of ruminal disappearance of straw was influenced by protein intake. Caton et al. (1988), Silva and Ørskov (1988), Coleman and Wyatt (1982), Beaty et al. (1994) and Hunt et al. (1989) all measured increases in ruminal degradability of forages with protein supplementation.

There was no effect of day when bags were placed in the rumen on disappearance for Low-2, High-2, and High-3 treatments (Fig. 3.1). These results are consistent with those of Hunt et al. (1989) who found no difference in fibre degradability with grass hay when cottonseed meal was fed every 12, 24, or 48 hours.

3.4.3 Digestibility of diets

Major differences in estimated in vivo digestibility were observed depending upon which digesta marker was used (Table 3.6). Mean DM digestibilities, as derived from lignin, cobalt-EDTA and chromium-fibre markers, were 55, 64, and 70%, respectively, when the low protein concentrate was fed. The corresponding digestible energy contents of the diet were 9.8, 11.6 and 12.8 MJ kg^{-1}. According to NRC (1996) information average barley straw (70% of the diet) and barley grain (which comprised the majority of the concentrate) contain 7.4 and 16.1 MJ kg^{-1} digestible energy, respectively, thus the calculated energy content of the low protein diet was 10.0 MJ kg^{-1}. The expected digestibility of protein can be calculated from the formula: % digestible crude protein = 0.877 (% crude protein) – 2.64 (NRC 1984). Using this formula for the low and high protein diets (7.9 and 11.5% protein, respectively), the corresponding calculated digestible protein contents were expected to be 4.3 and 7.4% which corresponds to percentage digestibilities of 54 and 64%. The percentage digestibility of protein for the low and high diets were, respectively, 49 and 65; 60 and 71; and 66 and 75% when lignin, cobalt-EDTA, and chromium-fibre were used for estimation. On the basis of these estimates, we have confidence that the results obtained with lignin marker were satisfactory, even though there were some analytical problems associated with difficulties in filtering concentrates. We cannot explain the high digestibilities associated with the Co-EDTA and chromium-fibre markers, particularly for the animals fed concentrate daily, since both markers have been used successfully in studies with ruminant animals previously. Inherently we would prefer the use of lignin for this type of study since it is a constituent of both NDF and ADF in plant fibre. Digestibility estimates with this marker should thus not be affected by frequency of feeding or fecal sampling

regimen. In contrast, if frequency of concentrate feeding caused differences in digestibility between days, or if there was inadequate mixing within the digestive tract, external markers such as cobalt-EDTA and chromium-fibre could give variable results unless fecal samples taken on different days were composited exactly in proportion to the fecal dry matter produced.

Crude protein digestibilities were higher with the high protein diet (Table 3.6) as expected (see above discussion). In addition, digestibilities of DM, NDF, ADF and energy were improved with the high protein diets when lignin was used as a marker and there was a tendency for the digestible energy content to be influenced by protein with the cobalt-EDTA marker. Results of our study are confounded in that we cannot determine that extra starch in the low protein supplement fed either daily or alternate days resulted in a decrease in digestibility of DM, NDF, ADF, and energy, or that the extra protein in the high protein concentrate resulted in the increase in digestibility of the above parameters. Our results therefore confirm those of researchers such as Ortigues et al. (1990), Zorilla-Rios et al. (1991), Beck et al. (1992) and Fike et al. (1995) who measured improvements in the digestibility of straw associated with protein supplementation. Moreover, the improvement in digestibility is consistent with the 10% increase in ruminal degradability of straw which we observed at 24 h when straw was incubated in situ in steers with consuming the high protein diets (Table 3.5).

Frequency of feeding concentrate had no influence on the digestibility of DM, NDF, ADF, and energy (Table 3.6). Chase and Hibberd (1989) did not find that feeding a low or high level of maize daily or on alternate days influenced digestibility of grass hay in beef cows. Results of Collins and Pritchard (1992) support these findings in rams fed soybean meal or corn gluten meal every 24 or 48 hours. Coleman and Wyatt (1982)

also found that there was no difference in digestibility of native grass hay when cottonseed meal was fed daily, every other day, or every four days, but digestibility was depressed when small grains forage (wheat) was fed as a supplement every four days. In contrast, Beaty et al. (1994) found that decreasing feeding frequency from seven times per week to three times per week increased NDF digestibility 6.5% (51.1 vs. 54.4%).

There was no detectable difference in estimated digestibility of DM, NDF, ADF and energy depending upon whether fecal samples were collected on the day when concentrate was provided or on days when it wasn't (Table 3.7). This is not surprising for lignin since it is an internal marker and a constituent of ADF and NDF. However, for the water soluble Co-EDTA in particular, a fluctuation of marker concentration in the feces would be expected because of variable fecal excretion on days when concentrate was given and days when concentrate was not given. The mean residence time of straw in the rumen in this type of diet was 50 h in the study of von Keyserlingk and Mathison (1989). This appears to have been an adequate amount of time for mixing of the concentrate and grain portions of the diet with marker over a 2 or 3 d period before the material leaves the rumen. The observation that protein digestibility increased with time after feeding concentrate with the High-3 diet (Table 3.7 and A3) may indicate a reduced nitrogen excretion from the hind gut on days when concentrate was not fed.

3.5 Conclusions and Implications

Our study supports earlier research where it was found that straw DM intake was not influenced by supplementation frequency. Whether or not this was due to diet composition or animal behaviour cannot be ascertained from this study. Also in support of previous research, frequency of provision of concentrates had no effect on ruminal

disappearance of straw or on digestibilities of the diets. There were no differences between either ruminal disappearance of straw or diet digestibility when measured on days when supplement was fed and days when no supplement was fed. These results would therefore suggest that there should be no adverse effect on digestibility of the diet if concentrates are fed every second day rather than daily with straw-based diets. Although no significant differences were obtained, there were indications that voluntary intake may be reduced when concentrates are only fed every third day. Ruminal disappearances of straw were higher at 24 h with the high protein diet, and overall digestibility was increased with the high protein supplement but no increase in intake was detected.

3.6 References

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Table 3.1 Summary of treatments and days on which concentrates were fed (only first 6 days shown)

	Low protein concentrate		High protein concentrate		
Day of feeding cycle	Daily (Low-1)	Every 2nd day (Low-2)	Daily (High-1)	Every 2nd day (High-2)	Every 3rd day (High-3)
1	Fed	Fed	Fed	Fed	Fed
2	Fed	Not fed	Fed	Not fed	Not fed
3	Fed	Fed	Fed	Fed	Not fed
4	Fed	Not fed	Fed	Not fed	Fed
5	Fed	Fed	Fed	Fed	Not fed
6	Fed	Not fed	Fed	Not fed	Not fed

Table 3.2 Concentrate formulation and nutrient composition of straw and concentrates

Component	Straw	Low Protein Concentrate	High Protein Concentrate	SE[z]	Probability
Ingredients (% as-fed)					
Barley, dry rolled		97.8	64.4		
Canola meal			32.0		
Urea			1.4		
Fortified salt		2.0	2.0		
Vitamin ADE premix		0.2	0.2		
Composition (% of dry matter)					
Crude protein (%)	5.3c	14.1b	24.6a	1.02	<0.01
Neutral detergent fibre (%)	76.9			1.96	
Gross energy (MJ kg^{-1})	18.51b	18.68ab	18.92a	0.021	0.03

[z] Standard error of the mean is based on five observations per mean.
a-c Means not followed by the same letter differ (P<0.05).

Table 3.3 Effect of dietary regimen[Z] on mean daily dry matter intake over the experimental period

	Protein level fed daily or every 2 days contrast				Frequency of feeding Concentrate contrast				Individual treatments						
									Low protein		High protein				
	Low	High	SE[Y]	P[X]	1 day	2 days	SE[Y]	P[X]	1 day	2 days	1 day	2 days	3 days	SE[Y]	P[X]
Straw															
Kg	5.55	5.39	0.135	0.59	5.45	5.49	0.135	0.99	5.53	5.56	5.34	5.42	4.90	0.191	0.17
% body wt.	1.18	1.15	0.031	0.70	1.16	1.18	0.031	0.84	1.18	1.18	1.14	1.17	1.04	0.044	0.20
g $kg^{-0.75W}$	54.8	53.2	1.23	0.49	53.8	54.2	1.27	0.92	54.5	55.1	53.0	53.4	47.7	1.790	0.08
Concentrate															
Kg	2.27b	2.53a	0.070	0.03	2.52a	2.29b	0.070	0.04	2.45ab	2.10b	2.60a	2.48ab	2.21ab	0.098	0.03
% body wt.	0.48b	0.54a	0.014	0.02	0.54a	0.49b	0.014	0.04	0.52a	0.45b	0.55a	0.53a	0.47ab	0.020	0.02
g $kg^{-0.75}$	22.5b	25.0a	0.646	0.03	25.0a	22.5b	0.646	0.03	24.3ab	20.7b	25.8a	24.3ab	21.5b	0.913	0.02
Total															
Kg	7.82	7.92	0.145	0.52	7.97	7.78	0.145	0.30	8.00a	7.66ab	7.95a	7.90a	7.11b	0.205	0.05
% body wt.	1.66	1.69	0.032	0.43	1.69	1.66	0.032	0.49	1.70	1.63	1.69	1.70	1.51	0.045	0.56
g $kg^{-0.75}$	77.3	78.2	1.255	0.60	78.8	76.8	1.255	0.28	78.8a	75.8a	78.7a	77.7a	69.2b	1.779	0.02

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Standard error mean is based upon five animals per mean for individual treatments and ten animals per mean for contrasts.
[X]Probability.
[W]Metabolic body weight.
a,b Means not followed by the same letter differ ($P < 0.05$).

Table 3.4 Effect of whether concentrate was fed or not on straw dry matter intake for treatments in which concentrate was not fed daily[Z]

Item	Comparison of steers fed daily[Y]	Fed concentrate every 2 or 3 days				
		Day concentrate fed	Day after concentrate fed	2 days after concentrate fed	SE[X]	Probability
Low protein concentrate fed every 2 d						
Straw intake (kg d^{-1})	5.53	5.47	5.65		0.169	0.76
Straw intake (% of body weight)	1.18	1.16	1.20		0.036	0.76
Straw intake (g $kg^{-0.75}$ d^{-1})	54.6	54.1	56.0		1.710	0.74
High protein concentrate fed every 2 d						
Straw intake (kg d^{-1})	5.35	5.34	5.51		0.087	0.24
Straw intake (% of body weight)	1.14	1.15	1.19		0.018	0.23
Straw intake (g $kg^{-0.75}$ d^{-1})	53.0	52.6	54.2		0.839	0.23
High protein concentrate fed every 3 d						
Straw intake (kg d^{-1})	5.35	4.78	4.82	5.11	0.169	0.36
Straw intake (% of body weight)	1.14	1.02	1.02	1.09	0.036	0.36
Straw intake (g $kg^{-0.75}$ d^{-1})	53.0	46.3	46.9	49.8	1.693	0.35

[Z]Low or high protein concentrates fed daily, every 2 days, or every 3 days.
[Y]Compared with low protein concentrate fed daily and high protein concentrate fed daily for low and high protein concentrates, respectively.
[X]Standard error mean is based upon results from five animals per mean.

Table 3.5 Effect of dietary regimen[Z] on ruminal disappearance (%) of barley straw

Incubation time	Protein level fed daily Or every 2 days contrast				Frequency of feeding Concentrate contrast				Individual treatments						
									Low protein		High protein				
	Low	High	SE[Y]	P[X]	1 day	2 days	SE[Y]	P[X]	1 day	2 days	1 day	2 days	3 days	SE[Y]	P[X]
24 h	37.5b	41.1a	0.65	<0.01	39.5	39.0	0.65	0.60	37.0b	37.9b	42.1a	40.1ab	40.1ab	0.92	0.02
48 h	50.0	50.5	0.68	0.58	50.8	49.8	0.68	0.30	50.1	49.9	51.5	49.6	49.4	0.96	0.60
72 h	55.3	55.2	0.39	0.79	55.5	55.0	0.39	0.32	55.0	55.6	56.0	54.3	53.7	0.55	0.07
Mean[W]	47.6b	48.9a	0.44	0.05	48.6	47.9	0.44	0.27	47.4	47.8	49.9	48.0	47.8	0.62	0.10

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Standard error mean is based upon results from five animals per mean for individual treatments and ten animals per mean for contrasts.
[X]Probability.
[W]For repeated measures analyses SE and probabilities of treatment, time and treatment x time were 1.10, 0.12, <0.01 and < 0.01, respectively.
a, b Means not followed by the same letter differ (P < 0.05).

Table 3.6 Effect of dietary regimen[Z] on apparent digestibility of diets based upon barley straw as estimated by different digesta markers

Item	Protein level fed daily or every 2 days contrast				Frequency of feeding Concentrate contrast				Individual treatments						
									Low protein		High protein				
	Low	High	SE[X]	P[W]	1 day	2 days	SE[X]	P[W]	1 day	2 days	1 day	2 days	3 days	SE[X]	P[W]
Lignin marker															
DM[Y] (%)	54.7b	57.5a	0.76	0.04	55.9	56.1	0.76	0.91	54.4	55.0	57.8	57.2	58.7	1.08	0.08
NDF[Y] (%)	56.7	59.0	0.77	0.11	58.0	57.6	0.77	0.93	56.6	56.8	59.6	58.5	59.4	1.08	0.31
ADF[Y] (%)	42.9b	46.4a	0.56	<0.01	43.9	45.1	0.56	0.21	41.9b	43.9b	46.4a	46.4a	47.5a	0.80	<0.01
Protein (%)	48.7b	64.8a	1.54	<0.01	57.0	55.6	1.54	0.29	49.4b	47.9b	66.5a	63.4a	64.6a	2.18	<0.01
Energy (%)	53.0b	56.1a	0.80	0.02	54.4	54.5	0.80	0.96	52.7	53.3	56.6	55.7	57.3	1.14	0.07
DE[Y] (MJ kg^{-1})	9.8b	10.5a	0.15	0.02	10.1	10.1	0.15	0.92	9.8b	9.9b	10.6ab	10.4ab	10.7a	0.21	0.05
Cobalt marker															
DM[Y] (%)	63.8	65.2	0.47	0.20	64.7	64.2	0.47	0.89	63.1	63.4	65.4	65.0	63.1	0.67	0.33
NDF[Y] (%)	65.2	66.0	0.45	0.75	66.0	65.2	0.45	0.77	65.7	64.6	66.3	65.7	63.3	0.64	0.12
ADF[Y] (%)	54.4	56.1	0.76	0.31	55.6	55.3	0.76	0.51	54.3	54.4	55.9	56.2	53.0	1.08	0.39
Protein (%)	59.7b	71.3a	0.91	<0.01	66.3	64.2	0.91	0.08	60.6c	58.6c	73.1a	69.9ab	66.0b	1.28	<0.01
Energy (%)	62.4	64.0	0.45	0.10	63.5	62.9	0.45	0.74	62.8	62.1	64.4	63.8	61.8	0.64	0.22
DE[Y] (MJ kg^{-1})	11.6	11.9	0.08	0.06	11.8	11.7	0.08	0.69	11.7	11.5	12.0	11.9	11.5	0.11	0.18
Chromium marker															
DM[Y] (%)	70.1	69.5	0.98	0.87	68.9	70.6	0.98	0.39	70.0	70.2	67.7	71.0	69.4	1.38	0.84
NDF[Y] (%)	71.3	70.4	0.91	0.62	70.1	71.6	0.91	0.38	71.4	71.2	68.6	72.0	69.9	1.29	0.64
ADF[Y] (%)	62.2	61.4	1.39	0.81	60.3	63.2	1.39	0.25	61.7	62.8	58.5	63.7	61.1	1.96	0.73
Protein (%)	66.2b	75.1a	1.18	<0.01	70.3	70.6	1.18	0.71	66.6b	65.9b	75.0a	75.3a	73.8a	1.68	<0.01
Energy (%)	68.9	68.5	0.98	0.99	67.9	69.5	0.98	0.44	68.8	69.0	66.8	70.0	68.3	1.38	0.87
DE[Y] (MJ kg^{-1})	12.8	12.8	0.18	0.75	12.6	13.0	0.18	0.40	12.8	12.8	12.4	13.1	12.7	0.25	0.80

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Abbreviations: DM = dry matter, NDF = neutral detergent fibre, ADF = acid detergent fibre, and DE = digestible energy.
[X]Standard error mean is based upon results from five animals per mean for individual treatments and ten animals per mean for contrasts.
[W]Probability.
a, b Means not followed by the same letter differ ($P < 0.05$).

Table 3.7. Effect of day of obtaining fecal samples on estimated apparent vivo digestibility in steers on different dietary regimens[Z] as estimated with the lignin marker

Item	Daily fed comparison[X]	Fed concentrate every 2 or 3 days				
		Day concentrate Fed	Concentrate not fed for 1 d	Concentrate not fed for 2 d	SE[W]	Probability
Low protein concentrate fed every 2 d						
Dry matter (%)	54.4	54.3	55.6		0.90	0.57
NDF[Y] (%)	56.6	56.6	57.0		0.74	0.91
ADF[Y] (%)	41.9	43.6	44.2		0.67	0.14
Protein (%)	49.4	45.6	50.3		2.91	0.73
Gross energy (%)	52.7	52.5	54.1		0.99	0.54
Digestible energy (MJ kg^{-1})	9.8	9.8	10.0		0.18	0.55
High protein concentrate fed every 2 d						
Dry matter (%)	57.8	56.9	57.5		1.05	0.71
NDF[Y] (%)	59.6	58.3	58.7		1.12	0.82
ADF[Y] (%)	46.4	46.1	46.6		1.26	0.81
Protein (%)	66.5	62.2	64.6		1.80	0.40
Gross energy (%)	56.6	55.4	56.0		1.08	0.70
Digestible energy (MJ kg^{-1})	10.6	10.3	10.4		0.20	0.69
High protein concentrate fed every 3 d						
Dry matter (%)	57.8	58.7	59.2	58.1	0.94	0.72
NDF[Y] (%)	59.6	60.1	59.8	58.7	1.10	0.64
ADF[Y] (%)	46.4	48.2	48.0	46.3	1.40	0.60
Protein (%)	66.5ab	60.8b	64.5ab	68.5a	1.69	0.03
Gross energy (%)	56.6	57.3	57.5	56.9	0.90	0.89
Digestible energy (MJ kg^{-1})	10.6	10.7	10.7	10.6	0.17	0.88

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Abbreviations: NDF = neutral detergent fibre, and ADF = acid detergent fibre.
[X]Compared with low protein concentrate fed daily and high protein concentrate fed daily for low and high protein concentrates, respectively.
[W]Standard error mean is based upon results from five animals per mean for individual treatments and ten animals per mean for contrasts.
a, b Means not followed by the same letter differ ($P < 0.05$).

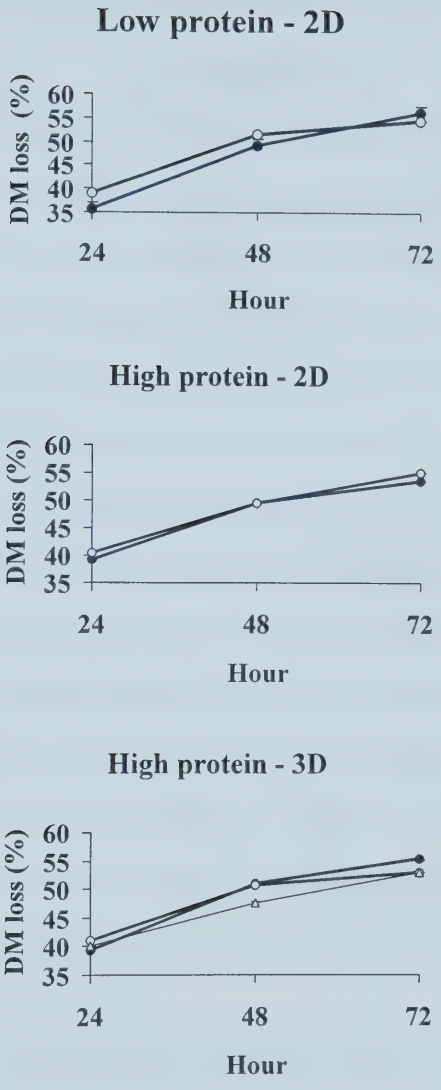

Fig. 3.1 Comparison of the effect of day of concentrate feeding (with 1 day (o) and 2 days after feeding concentrate (), on ruminal disappearance of straw dry matter. 2D and 3D refer to diets in which concentrate was given every second or third day, respectively. Pooled standard errors are 1.35, 1.36 and 2.08 for low protein -2D, high protein - 2 D and high protein - 3 D, respectively.

CHAPTER 4

Effect of Frequency of Concentrate Feeding on Ruminal pH and Metabolites

4.1 Introduction

Straw from cereal grains is an inexpensive feed resource for over-wintering beef cows. Production of wheat, barley and oats in Alberta in 2000 was over 13.3 million tonnes (AAFRD 2001). Using the kg yield of residue per kg grain produced (wheat 0.9 kg; barley 0.64 kg; oats 0.9 kg) used by AAFRD (1999) there would potentially be 10 million tonnes of straw available for animal consumption. There would be enough straw to feed approximately 5.6 million head of cattle (590 kg; consuming 1.5% body weight) for 200. That is 2 times the Alberta beef cowherd.

The concern with feeding straw are its low voluntary intake, low protein content, poor digestibility, low mineral and vitamin concentrations, and slow passage rate (Anderson 1978). As a result, straw diets must be supplemented with concentrates or high quality forages to meet the nutritional requirements of wintering beef cows. Supplemental feeds are costly. In addition, provision of supplemental feeds increase labour and equipment costs, especially in cold Canadian winters, thus there is interest in the effects of reducing frequency of providing supplemental feeds to less than once daily.

The prevailing opinion among nutritionists is that supplemental feed should be provided at least once daily to prevent severe fluctuations within the rumen and thus provide a more hospitable environment for ruminal microorganisms. However reducing supplementation frequency did not significantly impact rumen environment or metabolism in studies of Chase and Hibberd (1989), Collins and Pritchard (1992), and Beaty et al. (1994). McIlvain and Shoop (1962) found no difference in live weight gain

between grazing steers fed cottonseed cake daily and every third day. Some research even suggests that feeding supplements less than once daily can be beneficial. Thus Pate and Crockett (1971) reported that steers fed a high protein supplement every third day actually gained more weight than those fed the same supplement daily. Similarly, Huston et al. (1999) found that supplement and forage intakes were more variable within groups fed supplements once daily compared to those fed supplements less frequently. Other research is not as favorable for less frequent feeding of supplemental feeds. Thus Beaty et al. (1994) found that decreasing concentrate supplementation frequency increased winter weight loss when straw-based diets were fed.

The objective of this study was to investigate the hypothesis that feeding protein/grain supplements daily results in a more stable ruminal environment than feeding supplements on alternate days or every third day. Further it was hypothesized that when straw-based diets are fed that provision of higher protein supplements will, with the exception of ruminal ammonia concentrations, result in more stable rumen environments and enhanced digestion within the rumen.

4.2 Materials and Methods

4.2.1 Animals and Feed

Five crossbred steers (465±30 kg) were used in a 5 x 5 Latin square design experiment conducted at the Laird McElroy Environmental and Metabolic Center, University of Alberta Edmonton Research Station, Edmonton, Alberta, Canada. Steers were fitted with 10 cm i.d. soft ruminal cannula (Bar Diamond, Parma, ID) at least 90 days before the experiment as described previously (Chapter 3). Steers were maintained indoors in individual 3 m x 3 m pens with continuous lighting, and an average ambient temperature of 17°C throughout the experiment. All animals were

cared for in accordance with the guidelines of the Canadian Council of Animal Care (1993), and under the advice of the Faculty of Agriculture, Forestry, and Home Economics Animal Policy and Welfare Committee.

All steers were offered barley straw (Lacombe, six-row) ad libitum along with five different supplemental concentrate treatments. Concentrate treatments were as follows: 1) low protein concentrate fed daily (Low-1), 2) low protein concentrate fed every second day at two times the daily rate (Low-2), 3) high protein concentrate fed daily (High-1), 4) high protein concentrate fed every second day at two times the daily rate (High-2), 5) high protein concentrate fed every third day at three times the daily rate (High-3). Rate of concentrate supplementation was calculated as 30% of the total as-fed intake of the previous week. Steers were fed once daily at 0900h. Dietary treatments and procedures have been described more completely in Chapter 3.

4.2.2 Measurement of Ruminal pH, Ammonia, Lactic Acid and Volatile Fatty Acids

Rumen fluid samples were taken at 0, 3, 9, 13, 21 and 24 hours after supplementation. Approximately 100 mL of rumen fluid was obtained using a 60-mL syringe attached to a polyethylene tube weighted with a stainless steel rumen bullet. The pH was measured immediately upon sampling with a pH meter (Expandomatics SS2, Beckman Instruments, Fullerton, CA). Rumen samples for lactate and volatile fatty acid (VFA) analysis were prepared by adding 1 mL of 25% phosphoric acid to 4 mL of rumen fluid. Samples for ammonia determination were saved in a separate vial. All samples were frozen at -20°C until they could be analyzed.

Lactic acid and VFA analyses were performed by gas-liquid chromatography using a 30 m stable wax DA glass capillary column in a Varian Model 3600

chromatograph (Varian, Walnut Creek CA) according to the methods described by Khorasani et al. (1996) (Appendix A).

Ammonia-N was analyzed according to the method of Fawcett and Scott (1960).

4.2.3 Statistical Analysis

Ruminal pH and lactate, VFA and ammonia concentrations were analyzed as a 5 x 5 Latin Square design using the GLM procedure of SAS (SAS Institute, Inc. 1988). Treatments (n = 5), periods (n = 5) and animals (n = 5) were the main sources of variation. Means were separated using the Student-Newman-Keul's test (SAS Institute, Inc. 1988). Comparisons between feeding concentrates with different protein concentrations and feeding concentrates daily or on alternate days were obtained by using contrast statement in SAS (SAS Institute, Inc. 1988). Mean metabolite concentrations and pH were determined across days and data were subjected to statistical analysis for each of the five sampling times. Also, a single mean value across all days and sampling times for each animal and each period was used in statistical analysis. Ruminal parameters were also examined by a repeated measures analysis (SAS Institute, Inc. 1988), with time as the repeated measure. The GLM procedure of SAS (SAS Institute, Inc. 1988) was also used to determine the effect of day of concentrate feeding on mean pH, VFA, lactate, and ammonia-N concentrations within each dietary treatment for those treatments in which concentrates were not fed daily. The sources of variation in this analysis were animal and treatment.

4.3 Results

4.3.1 Ammonia Concentrations

The mean ruminal ammonia concentration for all treatments and times was 2.70 mM (Table 4.1). Concentrations peaked ($P < 0.01$) 3 h after feeding and there was a diet x time interaction ($P < 0.01$). The highest ($P < 0.05$) concentration (9.8 mM) was observed 3 h post-feeding when the high protein supplement was provided daily (High-1 treatment), and the lowest (0.55 mM) 13 h post-feeding when the low protein supplement was provided on alternate days (Low-2 treatment).

When the high protein concentrate was fed daily or every second day mean ruminal ammonia concentrations were twice ($P < 0.01$) concentrations when the low protein concentrate was fed (3.29 vs. 1.64 mM), with statistical differences being maintained at all sampling times except 15 h after feeding (Table 4.1). There was a trend ($P < 0.1$) for overall ruminal ammonia concentrations to be 14% lower in steers fed concentrate every second day (2.28 mM) in comparison with those fed concentrates daily (2.65 mM), although such a trend was not detected between 3 and 15 h after feeding (Table 4.1). Peak ammonia concentrations were 9.8, 7.6 and 6.4 mM for steers fed the High-1, High-2 and High-3 diets, respectively (Table 4.1). Surprisingly, there was less variation in mean ruminal ammonia concentrations averaged over 3 days when concentrate was fed every 3 days (High-3 diet) than when concentrate was provided daily (Table 4.1).

Ruminal ammonia concentrations peaked 3 h after feeding whether concentrate was provided or not (Fig. 4.1; Table A4, A7, A10). There were no differences in either overall mean (1.38 vs. 1.80 mM) or peak (3.46 vs. 3.36 mM) ruminal ammonia concentrations on days when concentrate was provided compared with days when concentrate was not provided for the Low-2 diet (Fig. 4.1, Table A4). However at 13 h after feeding, ruminal ammonia concentrations were four times higher ($P < 0.01$) on

days when steers were not fed concentrate than on days when steers were fed concentrates (0.89 vs. 0.21 mM; Table A4). In contrast on days when concentrate was fed with the High-2 diet, overall and peak ruminal ammonia concentrations were 173 and 347% ($P < 0.05$), respectively, of values on days when concentrate was not fed (Fig. 4.1; Table A7). When the high protein supplement was fed every third day (High-3 diet), mean ruminal ammonia concentrations were 5.1, 2.6 and 2.1 mM ($P = 0.01$) on day 0, 1 and 2 after concentrate feeding, respectively (Fig. 4.1; Table A10). On this dietary regiment the highest ammonia concentration (11.2 mM at 3 h after feeding) occurred on the day steers were fed concentrate, and this concentration was 287% ($P = 0.01$) of the concentration on days when no concentrate was fed (3.9 mM both days). There were no differences in the overall ruminal ammonia concentrations between the first and second day after concentrate was provided when the High-3 diet was fed or at any of the sampling times (Table A10).

4.3.2 Ruminal pH

Overall ruminal pHs were 6.90, 6.87, 6.86, 6.90 and 6.86 for the Low-1, Low2, High-1, High-2, and High-3 diets, respectively (mean 6.88; Table 4.1). No differences in pH were detected between individual dietary treatments, nor did dietary protein levels or frequency of providing concentrate influence ruminal pH (Table 4.1). Ruminal pH, was however, influenced by time after feeding ($P < 0.01$) and there was a diet x time interaction ($P = 0.1$). The lowest pH (mean 6.62) occurred 13 h after feeding and the highest (mean 7.03) just before feeding.

The mean daily pH on days when concentrate was fed was lower ($P < 0.01$) than the pH on days when no concentrate was fed for Low-2 (6.75 vs. 6.99), High-2 (6.72 vs. 7.07), and High-3 (6.63 vs. 6.93 and 7.03) diets (Fig. 4.1; Tables A4, A7 and A10).

4.3.3 Lactate concentrations

The mean concentration of lactic acid in the rumen of the steers was 0.22 mM (Table 4.1). Dietary regimen, time and diet x time did not influence lactate concentrations (P = 0.48, 0.11 and 0.65, respectively), nor were any differences detected between treatments at different times after feeding. However, the numerically highest mean daily lactate concentration (1.08 mM at 9 h after feeding) occurred when the high protein supplement was fed every third day (High-3 diet) whereas mean concentrations never exceeded 0.28 mM for any other treatment.

For the Low-2 diet, the mean lactate concentration on supplement feeding days was 167% (P = 0.02) of the concentration on days when supplement was not fed (Fig. 4.1; Table A4). With this dietary regimen, peak lactate concentrations (0.38 mM) occurred 15 h after concentrate feeding whereas peak concentrations (0.13 mM) occurred at 3 h after feeding on days when concentrates were not fed (Fig. 4.1; Table A4). With the High-2 diet, the mean ruminal lactate concentration was 0.24 mM on days when concentrate was fed compared with 0.13 mM (Fig. 4.1; Table 4.1) on days when no concentrate was fed (P = 0.11). Mean lactate concentrations were 0.82, 0.12 and 0.10 mM (P = 0.22) on the day concentrate was fed, the first day after feeding concentrate and the second day after feeding concentrate, respectively, when the High-3 diet was fed (Fig. 4.1; Table A10).

4.3.4 Volatile Fatty Acid Concentrations

4.3.4.1 Total volatile fatty acids

The mean concentration of volatile fatty acids (VFA) in the rumen across all treatments was 66.7 mM. Dietary protein levels had no influence on VFA concentrations when concentrates were fed daily or on alternate days, nor were differences detected

between the five individual treatments (Table 4.2). However, mean VFA concentrations were 8% lower (P = 0.04) in the rumen of steers fed concentrate every second day than in steers fed concentrates daily (65.1 vs. 70.4 mM; Table 4.2). Ruminal VFA concentrations were affected (P < 0.01) by time after feeding, with peak concentrations being observed 9 to 13 h after feeding. There was no diet x time interaction on this parameter.

With the Low-2 diet, total VFA concentrations were higher (P < 0.01) on days when concentrate was fed (72.7 mM) than on days when no concentrate was fed (57.5 mM, and concentrations were much more variable (Fig. 4.2; Table A5). On days when concentrate was not fed, the highest VFA concentration was observed at 0 h whereas peak VFA concentration was at 9 to 13 h when concentrate was fed. Similar results were obtained with the High-2 diet (Fig. 4.2; Table A8). With the High-3 diet, overall VFA concentrations were 77.4, 65.5 and 56.3 mM on the day concentrate was fed, first day after feeding, and second day after feeding, respectively (P = 0.03; Fig. 4.2; Table A11). For this diet, the differences in VFA concentrations between the day concentrate was fed and the day after occurred at all sampling times except 9 h after feeding. VFA concentrations were lower (P < 0.05) at 0 and 3 h on the second day compared with the first day after feeding concentrate.

4.3.4.2 Acetic and propionic acids

The mean concentrations of acetic and propionic acids in this experiment were 47.6 and 12.3 mM, respectively. Dietary protein intake did not influence either overall acetic or propionic acid concentrations in the Low-1, Low-2, High-1 and High-2 diet comparisons (P = 0.16 and 0.69, respectively; Table 4.2). Feeding concentrate once every second day rather than daily reduced (P = 0.02) overall acetic acid concentrations

by 8% from 50.8 to 46.6 mM when these diets were compared but had no influence on propionic acid (Fig. 4.2; Table 4.2). The alternate-day feeding regimen reduced ($P < 0.1$) mean acetic acid concentrations at all times except for 9 and 13 h but did not influence propionic acid concentrations at any sampling time. Differences between individual treatments were only observed at 24 h and for the mean concentration for acetic acid, whereas no differences between the five treatments were detected for propionic acid. Acetic and propionic acid concentrations peaked ($P < 0.01$) at 9 and 13 h after feeding (Table 4.2).

When Low-1, Low-2, High-1 and High-2 diets were fed, ruminal concentrations of acetic and propionic acid were higher ($P < 0.05$) on days when concentrate was offered than on days when it was not for all times after 3 h from feeding (Fig. 4.2; Table A5 and A8). With the High-3 diet, mean ruminal concentrations of acetic acid were 52.4, 46.5 and 41.0 ($P = 0.07$) on the 0, 1 and 2 day after concentrate feeding, respectively (Fig. 4.2; Table A11). Corresponding values for propionic acid were 15.2, 12.5 and 10.2 ($P < 0.01$; Fig. 4.2; Table A11). For this diet, acetic acid concentrations were highest on the day concentrate was fed at 13, 21 and 24 h after feeding, whereas differences persisted at all sampling times for propionic acid.

4.3.4.3 Acetic:propionic acid ratios

The mean acetic:propionic acid ratio in this experiment was 3.98. Protein level in the diet of steers which were fed concentrate once daily or every second day had no influence on the acetic:propionic acid ratios in the rumen (Table 4.3). Frequency of feeding concentrate did, however, affect the acetic:propionic acid ratio, with the mean ratio being 7% higher ($P < 0.01$) in animals fed concentrate daily (4.25) in comparison with the when animals were fed concentrate every second day (3.97; Table 4.3). The

acetic:propionic acid ratio was lowest (3.82; $P < 0.01$) in steers fed concentrate every third day (High-3 diet), but ratios were similar to those in steers fed concentrates every second day with the exception of at 24 h where the mean ratio was less with the High-3 diet ($P < 0.05$; 3.99 vs. 3.78).

With the exception of 0 and 9 h after feeding, significant differences in the acetic:propionic acid ratios due to day of feeding concentrate could not be detected for the Low-2 diet (Fig. 4.3; Table A5). However, in all cases there was numerical agreement between results obtained with this diet and with the High-2 diet. With the High-2 diet mean acetic:propionic acid ratios were 3.8 on days when concentrate was fed in comparison with 4.2 on days when concentrates were not fed (Fig. 4.3; Table A8). When the High-3 diet was fed acetic:propionic acid ratios were 3.6, 3.8 and 4.1 ($P < 0.01$) on the day concentrate was fed, the day after concentrate was fed and the second day after concentrate was fed (Fig. 4.3; Table A11). After 3 h after feeding, however, acetic:propionic acid ratios in the rumen did not differ on the first or second day after the steers received concentrate.

4.3.4.4 Butyric, isobutyric, valeric and isovaleric acids

The mean concentration of butyric acid in the rumen of the steers was 5.0 mM (Table 4.3). When concentrates were fed daily or on alternate days, protein content of the diets had no influence on the concentration of this acid. Overall ruminal concentrations of butyric acid were 20% higher ($P = 0.01$) in steers fed concentrate daily than in steers fed concentrate every second day (Table 4.3). Concentrations were also higher ($P < 0.05$) in the daily fed animals at 0, 3, 9, 21 and 24 h but not at 13 h. Compared to days when concentrate was fed, overall butyric acid concentrations were 66, 56, and 59% ($P < 0.01$) of concentrations on days when concentrate was not fed for

the Low-2, High-2, and High-3 diets, respectively (Fig 4.3; Table A6, A9 and A12). With the High-3 diet, overall ruminal butyric acid concentrations on the second day after concentrate feeding were 54% of the concentrations on the days when concentrate was fed ($P < 0.01$; Table A12). Butyric acid concentrations were maximal ($P < 0.01$) at 13 h after feeding when concentrate was fed daily and at 0 h on the day after concentrate feeding when concentrate was fed on alternate days.

The mean concentration of isobutyric in the rumen of the steers in this experiment was 0.58 mM (Table 4.3). When concentrates were fed daily or on alternate days, protein content of the diets had no influence on the concentration of isobutyric at any time after feeding. When concentrates were fed daily or on alternate days, protein content of the diets had no influence on the concentration of isobutyric acids at any time after feeding. No differences in ruminal concentrations of isobutyrate were detected between animals fed concentrates daily and every second day (Table 4.3). Similarly, there were no differences between the five dietary regimens at any sampling time. Mean overall isobutyric acid concentrations on the day after feeding concentrate were 92, 100, and 143% of concentrations in animals fed concentrate daily for diets Low-2, High-2 and High-3, respectively (Fig 4.3; Table A6, A9 and A12). Only with the latter diet was the difference significant ($P < 0.01$). On the second day after feeding concentrate with the High-3 diet, isobutyric acid concentrations were similar to those found in animals fed concentrates daily (0.49 vs. 0.51 mM; Table A12).

The ruminal concentration of valeric acid was 0.52 mM in this experiment (Table 4.4). Protein content of the diet had no inluence on valeric acid concentrations when Low-1, Low-2, High-1 and High-2 diets were compared. Similarly, mean valeric acid concentrations were similar in steers fed concentrates daily and every second day,

although at 3 h after feeding concentrations were 28% higher ($P < 0.01$) in steers fed concentrate daily (Table 4.4). Mean overall valeric acid concentrations on the day after feeding concentrate were 62, 53, and 70% ($P < 0.05$) of concentrations in animals fed concentrate daily for diets Low-2, High-2 and High-3, respectively (Tables A6, A9 and A12). On the second day after feeding concentrate with the High-3 diet, valeric acid concentrations were 50% of concentrations ($P = 0.02$) in animals fed concentrates daily (Table A12).

The mean concentration of isovaleric acids in the rumen of the steers in this experiment was 0.75 mM (Table 4.4). When concentrates were fed daily or on alternate days, protein content of the diets had no influence on the overall concentration of isovaleric acid, but at 24 h after feeding the concentration of this acid was 15% higher in rumen of steers receiving the high protein diet (Table 4.4). There were no differences in isovaleric concentrations between animals fed concentrates daily or every second day, although there was a tendency ($P = 0.06$) for concentrations of this acid to be higher in daily fed animals at 3 h after feeding (0.80 vs. 0.70 mM; Table 4.4). There were no differences between the five dietary regimens at any sampling time (Table 4.4). Mean overall isovaleric acid concentrations on the day after feeding concentrate were 83, 89, and 119% ($P < 0.05$) of concentrations in animals fed concentrate daily for Low-2, High-2 and High-3 diets, respectively (Tables A6, A9 and A12). On the second day after feeding concentrate with the High-3 diet, isovaleric acid concentrations were similar to those found in animals fed concentrates daily (0.61 vs 0.67 mM; Table A12).

4.4 Discussion

4.4.1 Ruminal environment in relation to microbial requirements

Since barley straw contains a very high percentage of fibre (72% cell walls; NRC 1996), microbial activity should be optimized if intake and digestibility of straw-based diets are to be maximized.

It was not possible for us to measure all aspects of the ruminal environment but we have obtained information on ruminal pH and concentrations of ammonia, isobutyric acid, valeric acid and isovaleric acid which have all been shown to affect microbial growth and activity.

4.4.1.1 Rumen Ammonia

Overall ruminal ammonia concentrations increased by 100% when the higher protein concentrate was fed either daily or on alternate days (Table 4.1). This would be expected as the high protein supplement contained 74% more crude protein than the low protein supplement (24.6 vs. 14.1% CP) as a result of the addition of canola meal (Table 3.2). Overall protein concentrations for the low and high protein diets were 7.9 and 11.5% respectively.

In this study, ruminal ammonia levels peaked at 3-h post-supplementation in all dietary treatments whether supplement was provided or not (Fig. 4.1). Chase and Hibberd (1989) found similar results in beef cows fed low-quality grass hay supplemented with corn at a rate of 1.4 kg daily or 2.8 kg on alternate days, or 2.0 kg daily or 4.1 kg on alternate days. Similarly, Hunt et al. (1989) found that ruminal ammonia levels peaked at approximately 3 h post-supplementation in steers fed a grass hay diet and supplemented with soybean meal or cottonseed meal every 48 h. Beaty et al. (1994) also found that ruminal ammonia levels peaked at approximately 4 h post-supplementation in steers fed a wheat straw diet and provided with a soybean meal and sorghum grain supplement three times per week. In contrast to the results of our study,

where ruminal ammonia concentrations peaked even on days when no supplemental protein was supplied (Tables A4, A7 and A10), ruminal ammonia concentrations continued to decline throughout the day in studies of Hunt et al. (1989) and Beaty et al (1994). Differences between our results and those of Hunt et al. (1989) and Beaty et al. (1994) may be in part due to the following reasons. Ruminal ammonia levels in the study of Hunt et al. (1989) were highest at 0 h postfeeding than at any other time on days when no supplement was fed. Beaty et al. (1994) sampled at 4 h postfeeding versus our sampling at 3 h postfeeding, thus they may have inadvertently missed the peak in ruminal ammonia concentrations. Collins and Pritchard (1992) obtained similar results to Hunt et al. (1989) when soybean meal and corn gluten meal were provided every 48 hours to wethers fed corn stalk diets.

According to NRC (1996) the steers at maintenance required 544 g of degradable intake protein. Using their feed composition data the low and high protein diets would have provided 281 and 430 g of degradable intake protein respectively. This suggests that the ruminal ammonia concentrations would be lower than required for optimal microbial activity. Satter and Slyter (1974) suggested a minimum of 3.6 mM (5 mg dl^{-1}) of ruminal ammonia nitrogen is required to maximize growth of bacteria in the rumen. There is, however, a wide range (1.4 to 16.4 mM or 2 to 23 mg N 100 mL^{-1}) of ruminal ammonia concentrations that are considered to be optimal for microbial production in the rumen (Hunt et al. 1989). Optimal concentrations of ruminal ammonia can vary widely depending upon species and rumen conditions, and may in fact be higher for diets low in crude protein than for diets high in crude protein (Tamminga 1993). With the low protein concentrate in our study the mean the mean ruminal ammonia concentration was 1.64 mM (Table 4.1) and much lower concentrations were

measured on the day when concentrate was fed with the Low-2 diet (0.36 and 0.21 mM at 9 and 13h, respectively; Table A4). Therefore the low protein diet provided too little protein to support maximal microbial growth in the rumen and hence it would be expected that fibre digestion in the rumen would be reduced when the low protein concentrate was fed. This is evidenced in Chapter 3 where the 24 h ruminal DM degradability of straw was increased by 10% ($P < 0.01$) when the high protein concentrate was fed compared to when the low protein concentrate was fed (Table 3.5). Even when the high protein concentrate was provided mean ruminal ammonia concentrations were 3.6, 2.97 and 3.28 mM for diets High-1, High-2 and High-3, respectively, which would be marginally deficient for microbial requirements according to conservative guidelines of Satter and Slyter (1974). Thus there were times throughout the day and feeding cycle when concentrates were not fed daily when ruminal ammonia concentrations with the high protein concentrate were greatly below requirements. With the High-1, High-2 and High-3 diets, mean ruminal ammonia concentrations fell to as low as 1.58, 1.09 and 2.62 mM, respectively at 13 h after feeding concentrate (Table 4.1). At this time ammonia concentrations fell to as low as 0.9, 1.5 and 1.3 mM on days when concentrate was not fed with the High-2, High-3 (day after feeding concentrate), and High-3 (second day after feeding concentrate), respectively. This data would suggest that the high protein concentrate was also inadequate in terms of providing optimal ruminal ammonia concentrations. Another possibility for the low ruminal ammonia concentrations that we saw in this study is that microbial utilization of ammonia was such that it was not able to accumulate in the rumen.

It can therefore be concluded that ruminal ammonia concentrations were not sufficient for an optimal rate of digestion of fibre with these diets. The reductions in

digestibility which were noted with the low protein diet (Chapter 3) were thus to be expected. Further, our results would suggest that further marginal increases in digestibility might be achieved by supplementing the diet with a greater amount of protein even though the economics might not favor such supplementation.

4.4.1.2 Ruminal pH

Rumen pH is an important factor that influences fibre digestion in the rumen. Normal rumen pH is in the range of pH 6 to 7 and a pH less than 6 has been implicated in reduced fibre digestibility (Van Soest 1994). The NRC (1996) assumes that there is no digestion of fibre when ruminal pH falls below 5.7. In our study dietary treatment did not affect overall ruminal pH and the mean value of 6.88 was high enough for optimal fibre digestion. However, ruminal pH was lower on days when supplement was fed than on days when no supplement was provided. The lowest pH measured at any time of the day was a pH of 5.96 which was recorded 13 h after concentrate was fed in the High-3 feeding regimen (Table A10). The lowest pH reached with the Low-2 and High-2 regimens were pH 6.3 and 6.2 respectively (Table A4 and A7). The study of Beaty et al. (1994) supports these results since the lowest rumen pH of steers fed 3 times per week was just below pH 6.2 but never went below pH 6. Similarly, Chase and Hibberd (1989) found that lowest ruminal pH reached on days when supplement was provided were approximately 6.3 and 6.15 in heifers fed 2.8 kg or 4.1 kg of maize on alternate days. Collins and Pritchard (1992) noted that there was a diet x hour interaction for ruminal pH in animals fed a soybean meal or corn gluten meal supplement on alternate days, but found it to be of limited importance because all pH values were within the pH range of 6.3 to 6.8. Hunt et al. (1989) reported a low overall pH value of 6.0 for steers fed

cottonseed meal every 48 hours. They attributed this to the low buffering capacity of low quality grass forages.

There is no indication in our study that the low pH in the High-3 regimen reduced overall DM degradability. This is evidenced by the fact that no difference in overall degradability was detected between the High-1, High-2, or High-3 regimens (Table 3.5). Similarly, no difference was detected in DM degradability between days when supplement was fed and days when no supplement was provided (Table A1). Therefore, we can conclude that any possible effect of pH was so small that it could not be detected in our study.

4.4.1.3 Valeric and branched chain fatty acids

Valeric acid, and the branched chained isobutyric and isovaleric acids, have all been shown to be required by cellulolytic microorganisms in the rumen and, thus to influence microbial activity and growth in forage-based diets. Russell et al. (1992) demonstrated the need for amino acids and peptides for optimal bacterial protein synthesis. Substantial research has been done on the use of supplemental branched-chain and valeric acid as dietary supplements for dairy cows and their use use have found a beneficial effect of additional isoacids on milk production. These acids, however do not seem to be a limiting factor in the performance of growing beef cattle (Becht 1987; Andries et al. 1987; NRC 1996).

Mean concentrations over all treatments were 0.59, 0.52, and 0.76 mM for isobutyric, valeric, and isovaleric acids. These are low compared to the concentrations found by Zorilla-Rios et al. (1991), which were 1.15, 0.96, and 1.45 mM for isobutyric, valeric, and isovaleric acids in steers fed untreated barley straw with or without a SBM supplement. This indicates that the concentrations in our study may not support optimal

fibre digestion in the rumen, but this may not have a significant effect on the performance of non-lactating, pregnant cows.

Overall mean concentrations of isobutyric, valeric and isovaleric acid were not affected by supplementation frequency in our study (Tables 4.3 and 4.4). Similarly, Hunt et al. (1989) found no difference in concentrations of isobutyric, valeric, and isovaleric acid concentrations in steers fed cottonseed meal every 12, 24, or 48 hours. Collins and Pritchard (1992) also found that frequency did not affect isobutyric, valeric, and isovaleric acid concentrations in wethers fed cornstalk diets supplemented with soybean meal or corn gluten meal every 24 or 48 hours. However, those animals fed soybean meal had higher concentrations of isobutyric acid than those fed corn gluten meal even though the diets were designed to be isonitrogenous. This may be because the amino acid profile of the soybean meal is more favorable for branched chain fatty acid formation that that of the corn gluten meal.

It was a surprise that concentrations of these acids were not influenced by supplemental protein since branched chained fatty acids are formed when branched chained amino acids (e.g. valine, isoleucine and leucine) are deaminated (Yokoyama and Johnson 1988). Similarly, valeric acid can be formed from amino acids such as proline, lysine and arginine. It is possible that there was a shortage of corresponding amino acids (Flachowsky et al. 1988), or that fermentation was slow enough that the microbial population recycled enough isocarbon skeletons to meet requirements (Van Soest 1994). Flachowsky et al. (1988) found that animals fed concentrate-roughage diets (5.6 mmol L^{-1}) had a higher maximum concentration of branched chain fatty acids in the rumen fluid than animals fed roughage-only diets (3.4 mmol L^{-1}), which were in turn higher than in animals fed straw-starch-urea diets (2.1 mmol L^{-1}). The lower

branched chain fatty acid concentrations were attributed to a deficiency of the corresponding amino acids and high activity of cellulolytic microorganisms.

In conclusion, our data indicates that microbial activity and hence synthesis of microbial protein and fibre digestion may have been limited with all dietary treatments because of low concentrations of ammonia and branched chain fatty acids. In addition, there were times when ruminal pH was below the optimum for microorganisms. The lowered ruminal straw disappearance after 24 h incubation and the lowered DM and fiber digestibility with the low protein diets (Table 3.5) suggests that conditions were not optimal for maximum microbial fermentation, particularly with this diet.

4.4.2 Lactic acid and lactic acidosis

Lactate is a concern in high concentrate diets because it is associated with rumen acidosis. We expected that excessive lactate accumulations may be a problem when large amounts of concentrates are fed in the alternate day and every third day feeding regimen. Lactate is highly acidic and can result in a rapid decline in rumen pH in the presence of highly fermentable carbohydrates. As a result the microbial population shifts and fibre digestion is impeded. Lactic acid is ten times more acidic than VFAs, and a pH of 4 can result in rumenitis (Van Soest 1994).

Surprisingly, no differences were detected in ruminal lactic acid concentrations, with mean period values being 0.15, 0.16, 0.16, 0.18 and 0.34 mM for Low-1, Low-2, High-1, High-2, and High-3 respectively (Table 4.1). Thus even on days when 6.6 kg of concentrate were fed to steers assigned to the High-3 regimen there was no cause for concern of lactic acidosis. The highest concentration of lactic acid observed in the experiment was 3.09 mM (Table A10), which occurred in the High-3 regimen on the day when supplement was provided. Ruminal lactate levels greater than our experimental

maximum have been reported even when animals were fed no grain (Huntington et al. 1981). Lactic acid concentrations in the range of 20 to 100 mM were measured by Huntington and Britton (1979) in animals suffering from acute lactic acidosis and values as high as 220 to 320 mM have been recorded (Jensen and Mackey 1979). Lactic acid comprises 50 to 90% of total rumen acids in animals with severe lactic acidosis (Van Soest 1994) whereas they were less than 5% of the total in our experiment.

There are several possibilities as to why we never encountered a problem with lactic acidosis. The first is tha high fibre diets increase salivation, which transports urea and salivary buffers to the rumen, therefore helping maintain normal rumen pH. The major lactate producing organism, Streptococcus bovis, produces volatile fatty acids at normal rumen pH and only converts to lactate production when rumen pH falls below 6 (Van Soest 1994). Thus because the rumen pH was within normal range in our study this organism would probably have been producing proportionally more VFAs than lactate. Many of the microorganisms compete for the same substrate in the rumen (Van Soest 1994). Therefore, another possible reason could be that there was sufficient competition for substrate on the days that concentrate was fed as to prevent a proliferation of lactic acid producing bacteria.

4.4.3 Acetic, propionic and butyric acids

The main energy supply for the ruminant animal is the acetic, propionic and butyric acids derived from ruminal fermentation. Total VFA and acetic acid concentrations were not affected by protein content of the diet, but was higher ($P = 0.04$) in animals fed concentrate daily than in steers fed concentrate on alternate days (Table 4.2). Our results differ from those of Hunt et al. (1989) who fed a cottonseed supplement, Chase and Hibberd (1989) when beef heifers were fed a maize

supplement, and Collins and Pritchard (1992) when sheep were fed a corn gluten meal or a soybean meal supplement daily or on alternate days. All of these researchers reported that frequency of supplementation did not affect total VFA or acetic acid concentration.

Concentrations of propionic acid were not influenced by dietary protein concentration or frequency of supplementation (Table 4.2) in our study. Data of Hunt et al. (1989), Chase and Hibberd (1989) and Collins and Pritchard (1992) are in agreement with these results.

Butyric acid concentrations were not affected ($P = 0.57$) by protein intake but were reduced ($P = 0.01$) in animals fed concentrates on alternate days (Table 4.3). Fike et al. (1995) did not find an effect of protein supplementation in steers fed ammoniated wheat straw. Similarly, Zorilla-Rios et al. (1991) found no difference between unsupplemented steers and those fed 150 g d^{-1} soybean meal and wheat straw. However, butyric acid concentrations increased significantly when steers were provided with 500 g d^{-1} soybean meal. In contrast to the results of our study, Sunvold et al. (1991) found that supplementation of dormant bluestem range forage with protein supplements increased butyric acid concentrations. In agreement with Sunvold et al. (1991), Hunt et al. (1989) found an increase in butyric acid concentrations with protein supplementation, but found no effect of feeding cottonseed meal daily or on alternate days on butyrate concentrations in steers fed grass hay, which is in contrast to our results. Chase and Hibberd (1989) and Collins and Pritchard (1992) also found that there was no effect on butyric acid concentrations when supplement was fed daily or on alternate days.

Concentrations of acetic, propionic and butyric acid were higher on days when concentrates were fed than on days when they were not fed (Fig. 4.2 and 4.3). This would be expected since additional carbohydrate was fermented on days on which concentrates were given.

Acetate:propionate ratio was not influenced by protein content of the diet when concentrates were fed daily or on alternate days (Table 4.3). Zorilla-Rios et al. (1991) found no effect of protein supplementation (150 g d^{-1} or 500 g d^{-1}) on acetate:propionate ratio in steers fed wheat straw supplemented with 0, 150, or 500 g d^{-1} soybean meal. In contrast, Sunvold et al. (1991) found that the acetate:propionate ratio decreased significantly in steers fed dormant bluestem forage supplemented with protein versus those not supplemented at all. However, ratios were lower ($P < 0.01$) when concentrate was fed on alternate days than when it was fed daily in our study. This is in contrast to the results of Chase and Hibberd (1989) where providing a corn supplement daily or on alternate days to beef heifers fed low-quality hay did not influence acetate:propionate ratios. Decreased acetate:propionate ratios in animals fed supplement on alternate days in our study may indicate that energy status of the animal may be slightly improved compared to feeding supplement daily. Propionate is gluconeogenic and influences the glucose entry rate in the ruminant and can improve efficiency of energy use (Van Soest 1994).

Overall molar proportions of the major volatile fatty acids, acetate:propionate:butyrate, were 74:18:8, 74:19:7, 75:17:8, 74:19:7, and 72:20:8 for Low-1, Low-2, High-1, High-2, and the High-3 regimen. Over all treatments the ratio of these major VFA was 73:19:8. Owens and Goetsch (1988) gave 65:25:10 as an approximate ratio for roughage diets however this would be for a better quality forage

than used in our study. In agreement with our results, Collins and Pritchard (1992) observed an acetic:propionic:butyric acid ratio of 74:18:8 when wethers were fed a corn stalk diet supplemented with either soybean meal or corn gluten meal daily or on alternate days.

The acetate:propionate ratio was lower on days when supplement was provided than on days when no supplement was provided in our study. This would be expected because feeding concentrates shift fermentation end products towards propionate whereas feeding fibrous feeds shift fermentation end products towards acetate (Owens and Goetsch 1988).

4.5 Conclusions and Implications

Overall, rumen pH, ruminal ammonia, and volatile fatty acids were not largely influenced by frequency of supplementation. The exceptions were that total VFA, acetic acid and butyric acid concentrations were lower in animals fed concentrate every second day than in those fed concentrate daily. This also resulted in a lower acetic:propionic acid ratio in the steers fed concentrates on alternate days. Ruminal pH and lactic acid concentrations suggested that there was little likelihood of acidosis under this controlled feeding situation. However, when group feeding of cows is practiced some animals may receive considerably more concentrate than others which could pose a problem, particularly when concentrate is only fed every third day.

We observed that ruminal ammonia levels in steers fed the low protein concentrate (daily and alternate days) never reached levels sufficient for maximal microbial production in the rumen. Furthermore, even with the high protein diets ruminal ammonia concentrations fell below the optimal level on days when no supplement was fed. Therefore, supplemental dietary protein was required for maximal productivity, no

matter what the frequency of feeding. Surprisingly with the low protein diet, minimal ruminal ammonia concentrations were higher on days when concentrate was not fed than when it was fed. This effect could have arisen because of reduced microbial usage of ammonia or changes in urea recycling to the rumen. We obtained no evidence from the examination of the ruminal environment that the feeding of concentrate every third day was detrimental to the animals. However in light of the nonsignificant reduction in intake associated with this diet reported in Chapter 3, this practice cannot currently be recommended.

4.6 References

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Table 4.1 Effect of dietary regimen[Z] on ruminal ammonia and lactate concentrations and ruminal pH

Hours after feeding	Protein level fed daily or every 2 days contrast				Frequency of feeding concentrate contrast				Individual treatments						
	Low	High	SE[Y]	P[X]	1 day	2 days	SE[Y]	P[X]	Low protein		High protein			SE[Y]	P[X]
									1 day	2 days	1 day	2 days	3 days		
Ammonia[W] (mM)															
Overall	1.64b	3.29a	0.139	<0.01	2.65	2.28	0.139	0.08							
0	1.96b	2.57a	0.139	0.01	2.47	2.06	0.139	0.06	1.70b	1.59b	3.60a	2.97a	3.28a	0.20	<0.01
3	3.24b	8.71a	0.452	<0.01	6.45	5.50	0.452	0.16	2.10ab	1.82b	2.84a	2.30ab	2.34ab	1.01	0.04
9	0.81b	2.16a	0.192	<0.01	1.32	1.66	0.192	0.24	3.07c	3.41c	9.83a	7.59b	6.36b	0.64	<0.01
13	0.70	1.33	0.276	0.13	1.21	0.82	0.276	0.34	0.59b	1.02b	2.03a	2.28a	2.90a	0.27	<0.01
21	1.47b	2.38a	0.143	<0.01	2.12	1.72	0.143	0.07	0.85b	0.55b	1.58ab	1.09b	2.62a	0.39	0.02
24	1.70b	2.56a	0.157	<0.01	2.34	1.92	0.157	0.08	1.62bc	1.31c	2.62a	2.14ab	2.81a	0.20	<0.01
									1.96ab	1.43b	2.72a	2.39a	2.64a	0.22	<0.01
pH[W]															
Overall	6.88	6.86	0.015	0.89	6.88	6.89	0.015	0.82							
0	7.02	7.05	0.019	0.21	7.02	7.05	0.019	0.23	6.90	6.87	6.86	6.90	6.86	0.021	0.56
3	6.96	7.00	0.023	0.34	6.95	7.01	0.023	0.08	7.02	7.01	7.02	7.08	7.03	0.027	0.19
9	6.73	6.62	0.042	0.10	6.68	6.68	0.042	0.97	6.93	6.99	6.97	7.02	7.00	0.032	0.39
13	6.66	6.63	0.031	0.56	6.67	6.62	0.031	0.24	6.72	6.73	6.63	6.61	6.67	0.059	0.55
21	6.94	6.95	0.016	0.73	6.95	6.94	0.016	0.50	6.72	6.59	6.62	6.64	6.53	0.043	0.09
24	7.00	7.04	0.022	0.33	7.01	7.03	0.022	0.43	6.96	6.92	6.95	6.95	6.93	0.023	0.75
									7.02	6.98	6.90	7.08	7.01	0.031	0.22
Lactate[W] (mM)															
Overall	0.15	0.17	0.060	0.82	0.15	0.17	0.060	0.13	0.15	0.16	0.16	0.18	0.34	0.085	0.48
0	0.10	0.12	0.012	0.44	0.11	0.10	0.012	0.15							
3	0.13	0.19	0.026	0.12	0.14	0.18	0.026	0.68	0.09	0.11	0.13	0.11	0.15	0.017	0.19
9	0.22	0.28	0.300	0.88	0.24	0.25	0.300	0.15	0.13	0.13	0.16	0.23	0.21	0.036	0.21
13	0.23	0.18	0.050	0.51	0.18	0.24	0.050	0.09	0.20	0.22	0.27	0.28	1.08	0.425	0.55
21	0.14	0.14	0.022	0.98	0.13	0.14	0.022	0.62	0.21	0.26	0.15	0.22	0.34	0.072	0.43
24	0.10	0.11	0.019	0.75	0.11	0.11	0.019	0.81	0.13	0.13	0.13	0.14	0.16	0.032	0.98
									0.12	0.09	0.10	0.13	0.11	0.027	0.90

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Standard error mean is based upon five animals per mean for individual treatments and ten animals per mean for contrasts.
[X]Probability.
[W]For repeated measures analyses SE and probabilities of diet, time and diet x time were 0.48, <0.01, <0.01 and < 0.01; 0.051, 0.56, < 0.01, 0.10; and 0.209, 0.48, 0.11 and 0.65 for ammonia, pH and lactate, respectively.
a,b,c Means not followed by the same letter differ (P < 0.05).

Table 4.2 Effect of dietary regimen[Z] on ruminal total volatile fatty acid concentration and concentration of acetic and propionic acids

Hours after feeding	Protein level fed daily or every 2 days contrast				Frequency of feeding Concentrate contrast				Individual treatments						
									Low protein		High protein				
	Low	High	SE[Y]	P[X]	1 day	2 days	SE[Y]	P[X]	1 day	2 days	1 day	2 days	3 days	SE[Y]	P[X]
Total volatile fatty acids[W] (mM)															
Overall	66.4	69.2	1.60	0.24	70.4a	65.1b	1.60	0.04	67.6	65.1	73.2	65.2	66.4	2.27	0.13
0	58.2	60.4	1.97	0.45	61.2	57.4	1.97	0.19	59.2	57.2	63.3	57.5	59.9	2.78	0.57
3	59.0	61.1	2.01	0.47	63.6a	56.5b	2.01	0.03	61.8	56.1	65.3	56.9	59.3	2.84	0.20
9	76.4	82.7	2.68	0.12	82.8	76.2	2.68	0.11	78.8	73.9	86.8	78.5	72.8	3.79	0.14
13	78.2	82.3	2.27	0.22	81.9	78.6	2.27	0.31	77.4	79.0	76.5	78.1	79.9	3.20	0.32
21	66.6	67.4	2.07	0.78	69.7	64.4	2.07	0.10	68.5	64.8	78.9	64.0	65.6	2.92	0.47
24	59.9	61.0	1.64	0.63	63.2a	57.7b	1.64	0.03	60.2	59.5	66.2	55.9	60.7	2.31	0.09
Acetic acid[W] (mM)															
Overall	47.6	49.9	1.11	0.16	50.8a	46.6b	1.11	0.02	48.5ab	46.6b	53.1a	46.6b	46.6b	1.56	0.05
0	42.0	43.7	1.37	0.40	44.6	41.2	1.37	0.10	43.0	41.1	46.3	41.2	42.4	1.94	0.37
3	42.5	44.1	1.37	0.43	45.9a	40.7b	1.37	0.02	44.5	40.5	47.2	40.9	42.5	1.94	0.15
9	54.3b	59.6a	1.76	0.05	59.1	54.8	1.76	0.11	55.5	53.1	62.7	56.6	51.7	2.49	0.07
13	55.2	59.0	1.51	0.10	58.7	55.6	1.51	0.17	54.7	55.8	62.6	55.4	55.2	2.13	0.10
21	47.7	48.6	1.51	0.70	50.5a	45.8b	1.51	0.04	49.4	46.1	51.6	45.5	45.5	2.13	0.22
24	43.4	44.2	2.01	0.64	46.2a	41.5b	2.01	0.01	44.0ab	42.9ab	48.4a	40.0b	42.5ab	1.62	0.04
Propionic acid[W] (mM)															
Overall	12.0	12.2	0.39	0.69	12.1	12.1	0.39	0.91	11.9	12.1	12.4	12.1	12.6	0.55	0.90
0	10.1	10.4	0.38	0.55	10.1	10.4	0.38	0.67	9.9	10.3	10.4	10.5	11.1	0.53	0.60
3	10.3	10.5	0.46	0.73	10.7	10.1	0.46	0.39	10.6	10.0	10.8	10.2	10.9	0.65	0.83
9	14.4	14.8	0.70	0.65	15.0	14.2	0.70	0.45	14.8	14.0	15.2	14.5	13.9	0.99	0.88
13	14.8	15.0	0.62	0.76	14.6	15.2	0.62	0.58	14.3	15.3	15.0	15.1	15.6	0.88	0.88
21	12.0	11.9	0.43	0.87	11.9	12.0	0.43	0.79	11.9	12.1	11.8	12.0	12.6	0.61	0.92
24	10.4	10.6	0.36	0.74	10.5	10.5	0.36	0.93	10.0	10.8	10.9	10.3	11.5	0.51	0.32

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Standard error mean is based upon five animals per mean for individual treatments and ten animals per mean for contrasts.
[X]Probability.
[W]For repeated measures analyses SE and probabilities of diet, time and diet x time were 5.55, 0.13, <0.01 and 0.68; 3.83, 0.05, < 0.01, and 0.54; and 1.36, 0.90, <0.01 and 0.85 for total volatile fatty acids, acetic acid and propionic acid, respectively.
a,b,c Means not followed by the same letter differ (P < 0.05).

Table 4.3 Effect of dietary regimen[Z] on acetic acid to propionic acid ratio and concentrations of butyric and isobutyric acids

Hours after feeding	Protein level fed daily or every 2 days contrast				Frequency of feeding concentrate contrast				Individual treatments						
									Low protein		High protein				
	Low	High	SE[Y]	P[X]	1 day	2 days	SE[Y]	P[X]	1 day	2 days	1 day	2 days	3 days	SE[Y]	P[X]
Acetic: propionic acid ratio[W]															
Overall	4.06	4.16	0.060	0.27	4.25a	3.97b	0.060	<0.01	4.17ab	3.96b	4.34a	3.98b	3.82b	0.083	<0.01
0	4.20	4.25	0.049	0.50	4.42a	4.03b	0.049	<0.01	4.35a	4.06b	4.48a	4.02b	3.94b	0.070	<0.01
3	4.18	4.20	0.065	0.77	4.35a	4.04b	0.065	0.01	4.28ab	4.07ab	4.38a	4.02b	3.93b	0.092	0.02
9	3.91	4.14	0.099	0.13	4.00	4.04	0.099	0.79	3.83	3.99	4.18	4.10	3.83	0140	0.35
13	3.83	4.04	0.092	0.13	4.05	3.82	0.092	0.09	3.90	3.76	4.21	3.87	3.67	0.129	0.11
21	4.02	4.13	0.058	0.24	4.26a	3.88b	0.058	<0.01	4.16ab	3.89bc	4.37a	3.88bc	3.73b	0.083	<0.01
24	4.24	4.21	0.075	0.77	4.46a	3.99b	0.075	<0.01	4.48a	4.01b	4.44a	3.99b	3.78b	0.106	<0.01
Butyric acid[W] (mM)															
Overall	5.0	5.1	0.19	0.57	5.5a	4.6b	0.19	0.01	5.3	4.6	5.7	4.6	5.3	0.27	0.06
0	4.3	4.3	0.23	0.93	4.7a	4.0b	0.23	0.04	4.6	4.1	4.8	3.8	4.6	0.32	0.26
3	4.4	4.5	0.19	0.54	5.0a	3.9b	0.19	<0.01	4.8ab	3.9b	5.1a	3.9b	4.1ab	0.27	0.02
9	5.7	6.3	0.33	0.24	6.6a	5.3b	0.33	0.02	6.4	5.0	6.9	5.7	5.5	0.46	0.10
13	6.2	6.4	0.33	0.72	6.6	5.9	0.33	0.15	6.4	6.0	6.9	5.8	7.3	0.47	0.21
21	5.0	5.0	0.20	0.95	5.4a	4.7b	0.20	0.02	5.4	4.7	5.5	4.6	5.5	0.29	0.11
24	4.3	4.4	0.18	0.87	4.7a	4.0b	0.18	0.01	4.5ab	4.1ab	4.9a	3.8b	4.7ab	0.25	0.04
Isobutyric acid[W] (mM)															
Overall	0.59	0.60	0.02	0.73	0.61	0.58	0.02	0.36	0.60	0.58	0.62	0.57	0.57	0.03	0.75
0	0.58	0.62	0.03	0.32	0.60	0.60	0.03	0.97	0.58	0.59	0.63	0.62	0.61	0.04	0.89
3	0.61	0.62	0.02	0.24	0.60	0.55	0.02	0.11	0.57	0.64	0.63	0.56	0.53	0.03	0.23
9	0.57	0.61	0.03	0.40	0.62	0.54	0.03	0.07	0.63	0.56	0.60	0.52	0.47	0.04	0.09
13	0.60	0.59	0.04	0.77	0.61	0.58	0.04	0.45	0.61	0.59	0.61	0.56	0.54	0.05	0.79
21	0.61	0.62	0.03	0.87	0.63	0.59	0.03	0.34	0.63	0.59	0.64	0.59	0.62	0.04	0.88
24	0.57	0.61	0.02	0.34	0.60	0.58	0.02	0.76	0.57	0.58	0.63	0.59	0.62	0.04	0.71

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Standard error mean is based upon five animals per mean for individual treatments and ten animals per mean for contrasts.
[X]Probability.
[W]For repeated measures analyses SE and probabilities of diet, time and diet x time were 0.20, <0.01, <0.01 and <0.01; 0.67, and 0.06, < 0.01, 0.10; and 0.08, 0.75, <0.01 and 0.03 for acetic:propionic acid ratio, butyric acid and isobutyric acid, respectively.
a,b,c Means not followed by the same letter differ (P < 0.05).

Table 4.4 Effect of dietary regimen[Z] on concentrations of valeric and isovaleric acids

Hours after feeding	Protein level fed daily Or every 2 days contrast				Frequency of feeding concentrate contrast				Individual treatments						
									Low protein		High protein				
	Low	High	SE[Y]	P[X]	1 day	2 days	SE[Y]	P[X]	1 day	2 days	1 day	2 days	3 days	SE[Y]	P[X]
Valeric acid[W] (mM)															
Overall	0.51	0.52	0.02	0.61	0.53	0.50	0.02	0.40	0.51	0.50	0.54	0.50	0.54	0.03	0.78
0	0.39	0.40	0.02	0.70	0.38	0.41	0.02	0.34	0.35	0.42	0.40	0.40	0.44	0.06	0.47
3	0.54	0.60	0.03	0.18	0.64a	0.50b	0.03	0.01	0.59a b	0.50b	0.69a	0.51b	0.51b	0.04	0.03
9	0.65	0.68	0.04	0.59	0.70	0.64	0.04	0.24	0.71	0.60	0.70	0.67	0.61	0.06	0.53
13	0.60	0.61	0.04	0.85	0.62	0.59	0.04	0.56	0.61	0.59	0.63	0.59	0.63	0.06	0.96
21	0.46	0.45	0.03	0.89	0.44	0.47	0.03	0.38	0.44	0.47	0.43	0.47	0.57	0.04	0.11
24	0.39	0.39	0.02	0.98	0.39	0.38	0.02	0.84	0.38	0.40	0.41	0.37	0.51	0.04	0.11
Isovaleric acid[W] (mM)															
Overall	0.76	0.79	0.03	0.44	0.78	0.76	0.03	0.72	0.77	0.74	0.79	0.78	0.71	0.04	0.67
0	0.76	0.88	0.04	0.06	0.79	0.86	0.04	0.29	0.75	0.77	0.83	0.94	0.78	0.06	0.24
3	0.73	0.78	0.03	0.28	0.80	0.70	0.03	0.06	0.75	0.70	0.85	0.71	0.67	0.05	0.13
9	0.73	0.68	0.04	0.37	0.75	0.66	0.04	0.19	0.81	0.66	0.69	0.67	0.53	0.06	0.08
13	0.80	0.72	0.04	0.19	0.75	0.77	0.04	0.77	0.90	0.80	0.71	0.74	0.63	0.05	0.20
21	0.77	0.84	0.03	0.23	0.80	0.80	0.03	0.96	0.76	0.78	0.84	0.83	0.83	0.05	0.75
24	0.72b	0.83a	0.03	0.02	0.79	0.76	0.03	0.54	0.73	0.72	0.85	0.81	0.83	0.04	0.11

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Standard error mean is based upon five animals per mean for individual treatments and ten animals per mean for contrasts.
[X]Probability.
[W]For repeated measures analyses SE and probabilities of diet, time and diet x time were 0.08, 0.78, <0.01 and < 0.01; 0.10, 0.67, < 0.01, and <0.01 for valeric acid and isovaleric acid, respectively.
a,b,c Means not followed by the same letter differ ($P < 0.05$).

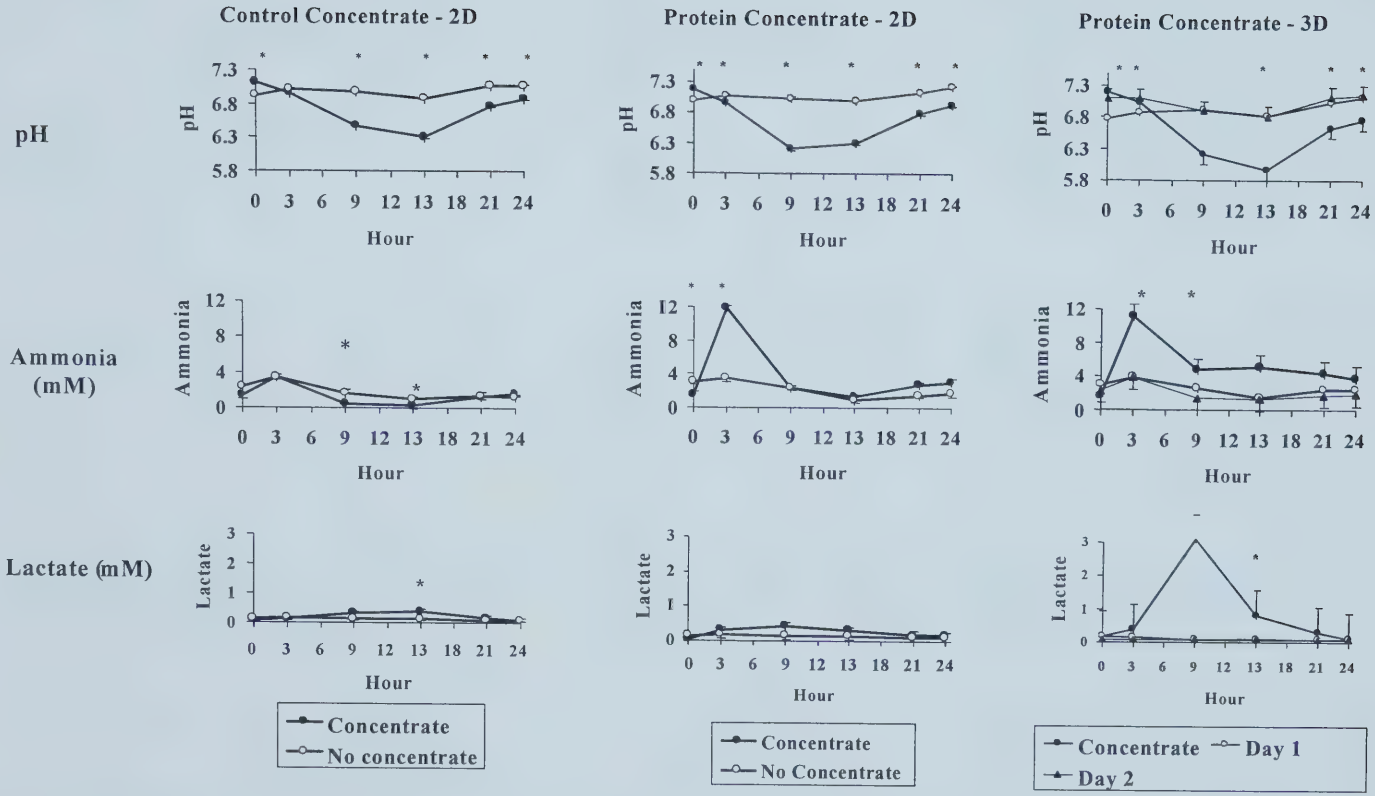

Fig. 4.1 Comparison of the effect of day of concentrate feeding (day 0) with days after concentrate feeding (day 1 or day 2), onruminal pH and ammonia and lactic acid concentrations. Control and protein concentrates refer to low and high protein concentrates, respectively. 2D and 3D refer to diets in which concentrate was given every second or third day, respectively. Vertical bars are pooled standard errors. * Indicate differences ($P < 0.05$) between days at that specific time.

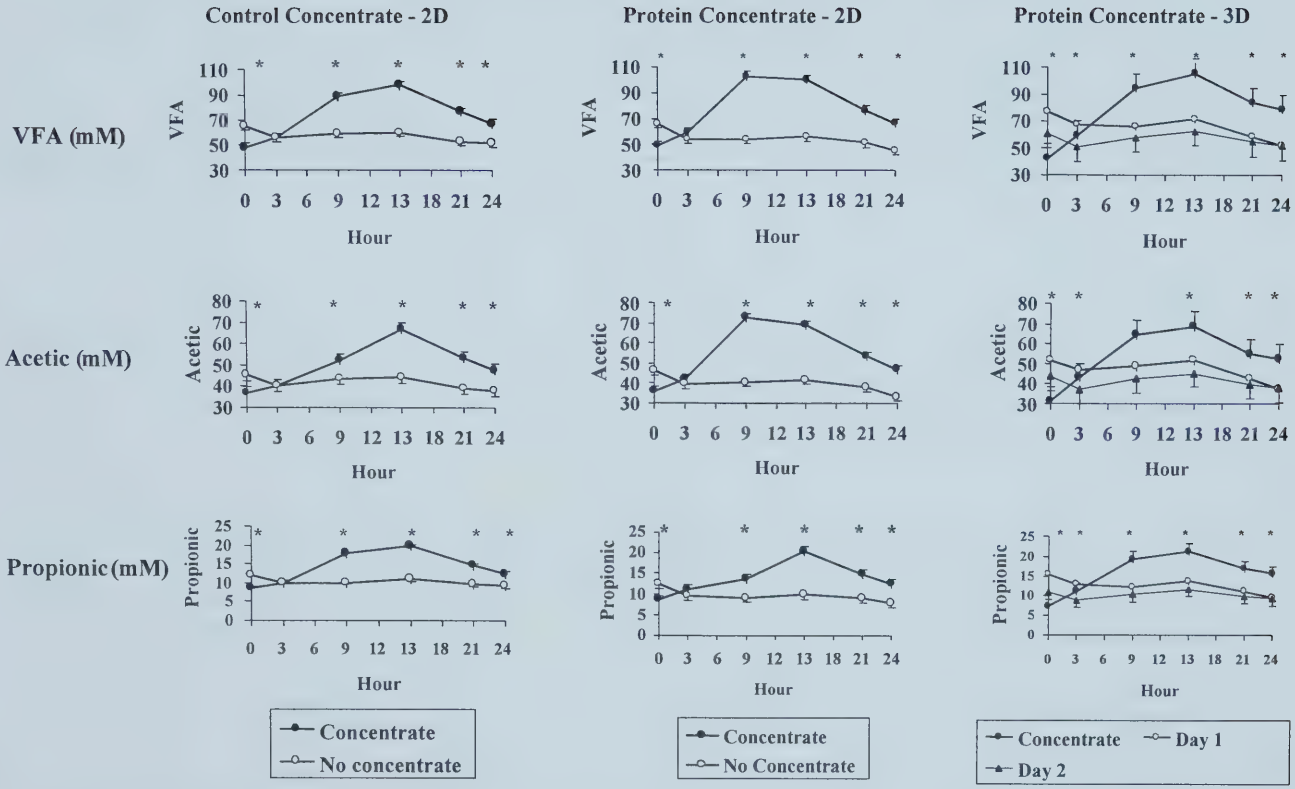

Fig. 4.2 Comparison of the effect of day of concentrate feeding (day 0) with days after concentrate feeding (day 1 or day 2), onruminal total volatile fatty acid, acetic acid and propionic acid concentrations. Control and protein concentrates refer to low and high protein concentrates, respectively. 2D and 3D refer to diets in which concentrate was given every second or third day, respectively. Vertical bars are pooled standard errors. * Indicate differences ($P < 0.05$) between days at that specific time.

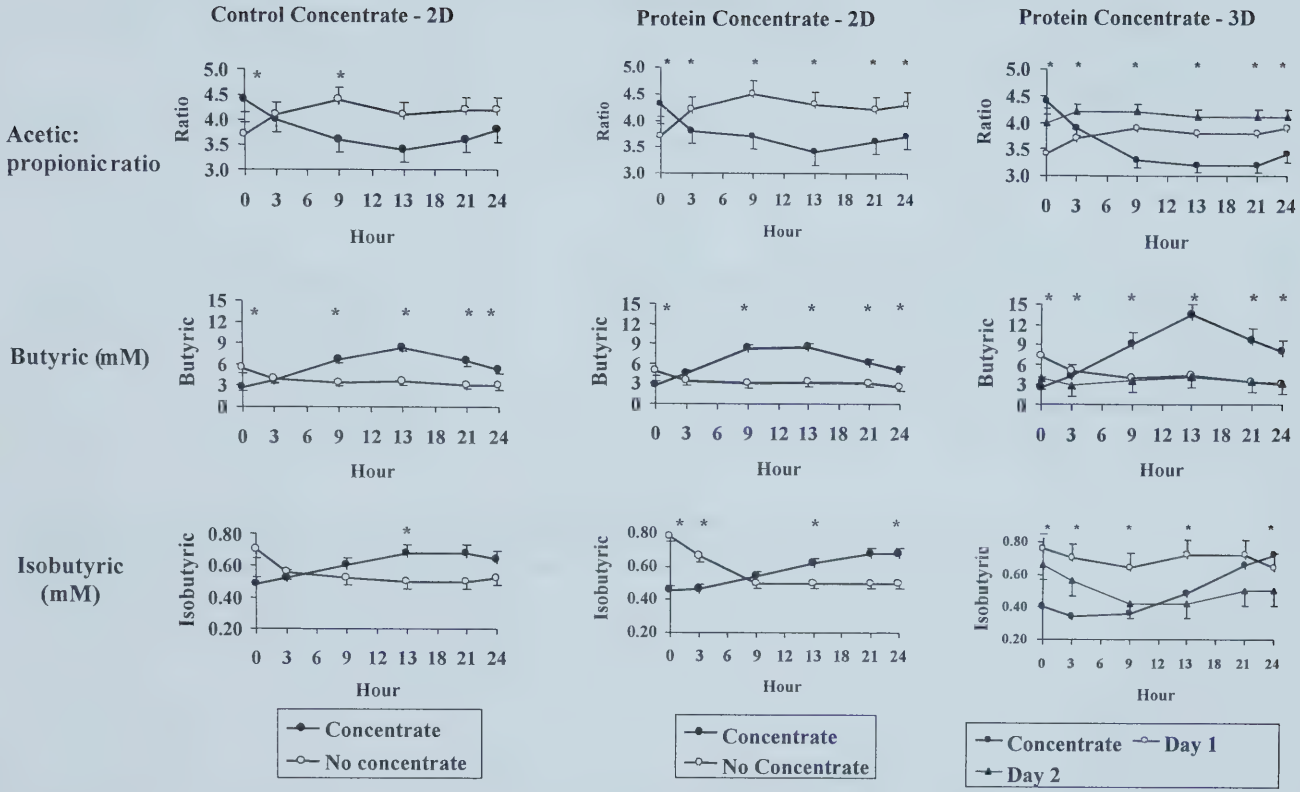

Fig. 4.3 Comparison of the effect of day of concentrate feeding (day 0) with days after concentrate feeding (day 1 or day 2), on ruminal acetic:propionic acid ratios and butyric and isobutyric acid concentrations. Control and protein concentrates refer to low and high protein concentrates, respectively. 2D and 3D refer to diets in which concentrate was given every second or third day, respectively. Vertical bars are pooled standard errors. * Indicate differences ($P<0.05$) between days at that specific time.

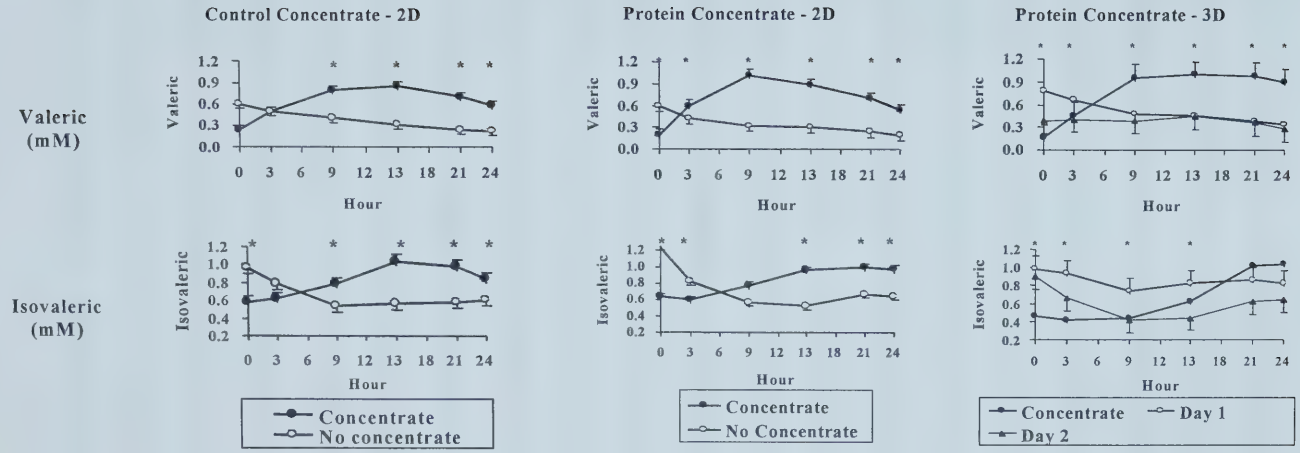

Fig. 4.4 Comparison of the effect of day of concentrate feeding (day 0) with days after concentrate feeding (day 1 or day 2), on ruminal valeric and isovaleric acid concentrations. Control and protein concentrates refer to low and high protein concentrates, respectively. 2D and 3D refer to diets in which concentrate was given every second or third day, respectively. Vertical bars are pooled standard errors. * Indicate differences ($P < 0.05$) between days at that specific time.

CHAPTER 5

General Discussion and Conclusions

Producers are constantly looking for alternative feeding strategies that may help reduce the cost of production. These reduced costs may be via a lower labour requirement, decreased equipment costs, utilization of cheaper low quality feedstuffs, or more efficient use of feed currently available. The objective of this study was to investigate the feasibility of an alternative feeding strategy by investigating its effects on voluntary intake, ruminal straw degradability and digestibility, and ruminal metabolites. Although we used steers to evaluate alternate day feeding strategies we feel that our results are transferable to the cow-calf producer. The steers were approximately 2 years of age during the trial and approaching maturity, thus digestibility, straw degradability, and rumen parameters would not be affected by steer age, thus results in our study should be indicative of results that would be obtained with cows. Van Soest (1994) has suggested that digestibility of diets would not be influenced by using steers.

Data in Chapter 3 shows that voluntary consumption of straw was 1.18, 1.18, 1.14, 1.17, and 1.04% of body weight for steers consuming the low protein concentrate daily, low protein concentrate on alternate days, high protein concentrate daily, high protein concentrate on alternate days, and high protein concentrate every third day ($P = 0.17$). Our results are in line with the results found by Johnson (1972), Mathison et al. (1981), Zorrilla-Rios et al. (1991), and Okine et al. (1993). Thus our hypothesis that frequency of supplementation would not affect straw DM intake was confirmed in this study, although the numerical decrease in intake with the latter dietary treatment warrants further investigation. Data in Chapter 3 also demonstrated that frequency of

supplementation did not have an effect on 24, 48, or 72-h ruminal degradability of straw or ultimate digestibility of the diet.

We also found that protein supplementation did not increase the intake of straw, which is supported by the results of Kay et al. (1968) and Alawa et al. (1988) but not others (.Sunvold et al. (1991; Beaty et al. 1994). Protein supplementation increased the rate of straw degradation in the rumen and improved dry matter and fibre digestion. This is consistent with results of other researchers (Coleman and Wyatt 1982; Hunt et al. 1989; Chase and Hibberd 1989; Collins and Pritchard 1992; Beaty et al. 1994)

Data in Chapter 4 demonstrated that overall ruminal ammonia concentrations were not influenced by supplementation frequency but that concentrations were higher when the high protein diet was fed. Ruminal ammonia concentrations did not differ between supplemented and non-supplemented days when the low protein supplement was fed. Although there may have been insufficient ammonia for optimal microbial digestion and growth, we found no evidence that this adversely affected intake, although it did affect digestibility. It is interesting to note that when the high protein supplement was fed every third day the ruminal ammonia levels on the second day without supplementation were not different than the previous day. This may be due to urea recycling or microbial death and breakdown of microbial nitrogen.

Our study shows that lactate levels and rumen pH did not differ between treatments for preotein level and frequency of supplementation. Total VFA were influenced by supplementation frequency. Our pH results are supported by the results of Hunt et al. (1989), Chase and Hibberd (1989), and Collins and Pritchard (1992) who also found that rumen pH, but their results are in contrast to ours in terms of total VFA. We therefore can conclude that under these controlled feeding conditions the feeding of

concentrate every second or third day rather than daily had no measured negative influence on the animal.

Molar proportions of acetic acid relative to propionic acid decreased when concentrate was provided every second day rather than daily. This contrasts with work of Chase and Hibberd (1989) and Collins and Pritchard (1992) who found that molar proportions of acetic acid were not affected by feeding frequency. There is evidence that the energetic efficiency of ruminant animals may be increased when the animal absorbs less acetic acid relative to propionic acid (Van Soest 1994). This aspect of our experiment deserves further research.

Molar proportions of isobutyric, valeric, and isovaleric acid concentrations were not affected by dietary regimen. Although such acids have been shown to be required by ruminal microorganisms, again there was no evidence that a deficiency of these resulted in reduced intake or digestibility.

The main objective of investigating new winter feeding strategies is the potential to improve the profitability of cow-calf enterprise. The potential for savings of implementing and alternate day feeding regime can be substantial. Okine (1999) evaluated the economics of feeding cows straw free choice supplemented with a silage-grain mixture either daily or on alternate days. Labour requirement for the first 78 days of the feeding period was 74.6 and 60.8 minutes per cow for the daily and alternate day regimes respectively. This translated to a labour cost of $18.17 and $14.82 per cow for the cows fed daily and those fed every other day respectively. Equipment costs for feeding and the removal of manure was $109.28 for the daily fed regime, and $101.09 for the alternate day regime over the 78 day period. By extrapolating this data to cover a 200 day feeding period and assuming feed costs would be the same for both the daily

and alternate day feeding regimes. The total labour requirement per cow is 3.2 and 2.6 hours (Table 5.1) for the daily and alternate day regimes respectively. The corresponding labour cost per cow for the 200 day feeding period would be $46.59 and $38.00 respectively, and equipment costs for feeding and manure removal would be $326.80 and $297.21 respectively. From these calculations we can see that there is a potential savings of $8.59 (18%) in labour costs, and $21.00 (7%) in equipment costs when an alternate day feeding regime is implemented. The total savings in labour and equipment costs when feeding on alternate days is $29.59 (9%) per cow for the 200 day feeding period. That is a substantial savings and warrants further investigation.

Our study therefore demonstrated that there was no measurable negative effect of reducing frequency of providing concentrates to every second day had a negative influence on intake or digestibility. Further, there was evidence of an improved acetic:propionic acid ratio with less frequent feeding which would be a positive effect. As a result, supplementing less frequently than once daily may be a viable alternative for many cow-calf producers. Large-scale production trials would be required to study the effects on liveweight and body condition changes, any potential impacts on calving percentage and fertility, and economics of feeding less frequently than daily.

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Table 5.1 Labour and equipment costs for animals fed daily compared to animals fed on alternate days

	Daily	Alternate Days	*Savings*
Labour/ cow (hours)	3.2	2.6	*0.6*
Labour cost/cow	$46.59	$38.00	*$8.59*
Equipment costs for feeding and manure removal	$280.21	$259.21	*$21.00*
Total	*$326.80*	*$297.21*	*$29.59*

APPENDIX A

Experimental Methods

Determination of Lactic Acid

Reference:

Khorasani, G. H., Okine, E. K. and Kennelly, J. J. 1996. Forage sources alter nutrient supply to the intestine without influencing milk yield. J. Dairy Sci. **79**:862-872.

One ml 25% phosphoric acid was added to 4ml of rumen fluid. The acidified rumen fluid was centrifuged at 2000 x *g* for 10 min. A 0.5-ml aliquot was taken, malonic acid was added as an internal standard, the solution was made basic with 3 N NaOH in methanol, and the mixture was evaporated to dryness. One ml 3 N HCl in methanol was added and the tube was stoppered and heated to 100°C for 25 min to dissolve the residue. The solution was allowed to stand until the NaCl precipitated and the resulting lactic acid ester was quantified by gas chromatography. The chromatograph was equipped with a 30-m (0.2 mm i.d.) capillary column. A split ratio of 50:1 was used with a column flow rate of 1 mL min^{-1} of nitrogen. The oven temperature was programmed to increase from 80 to 170°C at a rate of 25°C min^{-1} after an initial temperature hold of 0.5 min. The 170°C temperature was held for 3 min to complete the cycle.

Determination of Volatile Fatty Acids

Reference:

Khorasani, G. H., Okine, E. K. and Kennelly, J. J. 1996. Forage sources alter nutrient supply to the intestine without influencing milk yield. J. Dairy Sci. **79**:862-872.

One ml of 25% H_3PO_4 was added to 4 ml of rumen fluid. The acidified rumen fluid was centrifuged at 2000 x *g* for 10 min prior to analysis. Isocaproic acid (200 μL) was added to 1 ml of the acidified rumen fluid as an internal standard. A 1-μL sample of solution was injected into the chromatograph equipped with a 30-m (0.2 mm i.d.) capillary column. The flow rate of the carrier gas (helium saturated with formic acid) was 30 mL min^{-1}. The oven temperature was programmed to increase from 120 to 180°C at a rate of 10°C min^{-1} after an initial temperature hold of 1 min. The injector and detector temperatures were 170 and 200°C, respectively.

Table A1 Effect of day on which bags were placed in rumen on degradability (%) of barley straw dry matter

Incubation time	Concentrate Fed	Concentrate not fed for 1 d	Concentrate not fed for 2 d	SE[z]	Probability	Standard error and probabilities from repeated measures			
						SE	Diet	Time	Diet xTime
Low protein concentrate fed every 2 d									
Overall	47.0	48.4		0.78	0.27	1.35	0.27	<0.01	0.31
24 h	35.8	39.1		2.34	0.37				
48 h	49.1	51.6		0.85	0.10				
72 h	56.3	54.7		0.86	0.25				
High protein concentrate fed every 2 d									
Overall	47.4	48.3		0.79	0.47	1.36	0.47	<0.01	0.60
24 h	39.2	40.4		1.27	0.53				
48 h	49.4	49.4		0.79	0.96				
72 h	53.5	55.0		0.94	0.33				
High protein concentrate fed every 3 d									
Overall	48.5	48.0	46.8	1.19	0.53	2.06	0.53	<0.01	0.51
24 h	39.3	41.1	40.0	2.27	0.86				
48 h	51.0	50.8	47.6	1.40	0.15				
72 h	55.4	52.9	52.9	1.63	0.50				

[z]Standard error is based upon five animals per mean with data averaged over 2 or 3 days depending upon treatment. Three bags were incubated for each time.

Table A2 Effect of day of obtaining fecal samples on estimated apparent in vivo digestibility (%) in steers on different dietary regimens[Z] as estimated with the cobalt marker

Item	Daily fed comparison[X]	Fed concentrate every 2 or 3 days			SE[W]	Probability
		Day concentrate Fed	Concentrate not fed for 1 d	Concentrate not fed for 2 d		
Low protein concentrate fed every 2 d						
Dry matter (%)	64.1	63.8	63.1		1.52	0.88
NDF[Y] (%)	65.7	65.3	63.9		1.24	0.61
ADF[Y] (%)	54.3	55.2	53.7		1.39	0.75
Protein (%)	60.8	57.9	59.2		3.19	0.82
Gross energy (%)	62.8	62.4	61.7		1.63	0.90
Digestible energy (MJ kg^{-1})	11.7	11.6	11.5		0.31	0.90
High protein concentrate fed every 2 d						
Dry matter (%)	65.4	65.4	64.7		1.17	0.69
NDF[Y] (%)	66.3	66.2	65.2		1.11	0.55
ADF[Y] (%)	55.9	56.7	55.8		1.22	0.64
Protein (%)	73.1	69.6	70.2		2.27	0.86
Gross energy (%)	64.4	64.1	63.4		1.35	0.71
Digestible energy (MJ kg^{-1})	12.0	12.0	11.8		0.24	0.70
High protein concentrate fed every 3 d						
Dry matter (%)	65.4	61.8	63.7	63.8	1.07	0.38
NDF[Y] (%)	66.3	62.5	63.6	63.9	1.25	0.72
ADF[Y] (%)	55.9	51.9	53.7	53.5	1.52	0.66
Protein (%)	73.1	64.2	61.4	72.6	5.76	0.40
Gross energy (%)	64.4	60.5	62.2	62.7	1.10	0.38
Digestible energy (MJ kg^{-1})	12.0	11/3	11.6	11.7	0.21	0.37

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Abbreviations: NDF = neutral detergent fibre, and ADF = acid detergent fibre.
[X]Compared with low protein concentrate fed daily and high protein concentrate fed daily for low and high protein concentrates, respectively.
[W]Standard error mean is based upon results from five animals per mean for individual treatments and ten animals per mean for contrasts.
a, b Means not followed by the same letter differ ($P < 0.05$).

Table A3 Effect of day of obtaining fecal samples on estimated apparent in vivo digestibility (%) in steers on different dietary regimens[Z] as estimated by the chromium marker

Item	Daily fed comparison[X]	Fed concentrate every 2 or 3 days			SE[W]	Probability
		Day concentrate Fed	Concentrate not fed for 1 d	Concentrate not fed for 2 d		
Low protein concentrate fed every 2 d						
Dry matter (%)	70.0	69.6	70.7		1.30	0.84
NDF[Y] (%)	71.4	70.9	71.4		1.06	0.92
ADF[Y] (%)	61.7	62.4	63.2		1.30	0.84
Protein (%)	66.6	64.4	67.4		2.72	0.77
Gross energy (%)	68.8	68.4	69.6		1.37	0.84
Digestible energy (MJ kg^{-1})	12.8	12.7	12.9		0.25	0.85
High protein concentrate fed every 2 d						
Dry matter (%)	67.7	70.5	71.6		1.29	0.57
NDF[Y] (%)	68.6	71.6	72.3		1.19	0.72
ADF[Y] (%)	58.5	63.0	64.0		1.29	0.57
Protein (%)	75.0	74.2	76.4		2.25	0.52
Gross energy (%)	66.8	69.4	70.6		1.44	0.59
Digestible energy (MJ kg^{-1})	12.4	13.0	13.2		0.25	0.61
High protein concentrate fed every 3 d						
Dry matter (%)	67.7	67.6	69.2	71.4	1.33	0.18
NDF[Y] (%)	68.6	68.5	69.7	71.6	1.24	0.27
ADF[Y] (%)	58.5	59.1	60.7	63.4	1.77	0.28
Protein (%)	75.0ab	69.5b	73.3ab	78.5a	1.92	0.03
Gross energy (%)	66.8	66.4	67.9	70.6	1.40	0.16
Digestible energy (MJ kg^{-1})	12.4	12.4	12.7	13.2	0.26	0.17

[Z]Low or high protein concentrate fed daily, every 2 days, or every 3 days.
[Y]Abbreviations: NDF = neutral detergent fibre, and ADF = acid detergent fibre.
[X]Compared with low protein concentrate fed daily and high protein concentrate fed daily for low and high protein concentrates, respectively.
[W]Standard error mean is based upon results from five animals per mean for individual treatments and ten animals per mean for contrasts.
a, b Means not followed by the same letter differ ($P < 0.05$).

Table A4 Effect of alternate-day feeding of low protein concentrate on rumen pH, ammonia, and lactic acid concentrations

Hours after feeding	Concentrate fed	Concentrate not fed	SE[Z]	P[Y]	Repeated measures analysis			
					SE	Probability of diet	Probability of time	Probability of treatment x time
Rumen ammonia concentration (mM)								
Overall	1.38	1.80	0.14	0.45	0.34	0.09	<0.01	0.26
0	1.32	2.31	0.43	0.18				
3	3.46	3.36	0.67	0.93				
9	0.36b	1.68a	0.34	0.05				
13	0.21b	0.89a	0.09	<0.01				
21	1.25	1.37	0.34	0.81				
24	1.66	1.21	0.25	0.26				
pH								
Overall	6.75b	6.99a	0.01	<0.01	0.02	<0.01	<0.01	<0.01
0	7.11a	6.92b	0.03	0.01				
3	6.96	7.02	0.02	0.07				
9	6.48b	6.98a	0.05	<0.01				
13	6.30b	6.88a	0.09	0.01				
21	6.77b	7.07a	0.04	<0.01				
24	6.88b	7.08a	0.04	0.02				
Lactic acid (mM)								
Overall	0.20a	0.12b	0.02	0.02	0.06	0.06	0.02	0.06
0	0.10	0.11	0.02	0.68				
3	0.11	0.15	0.03	0.48				
9	0.32	0.13	0.11	0.29				
13	0.38a	0.13b	0.04	0.01				
21	0.18	0.09	0.03	0.13				
24	0.10	0.09	0.01	0.32				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.

[Y] Probability of difference

a,b Means not followed by the same letter differ.

Table A5 Effect of alternate-day feeding of low protein concentrate on total volatile fatty acids, acetic acid, propionic acid and acetic acid to propionic acid ratio

Hours after feeding	Concentrate fed	Concentrate not fed	SE[Z]	P[Y]	Repeated measures analysis			
					SE	Probability of diet	Probability of time	Probability of treatment x time
Total volatile fatty acids (mM)								
Overall	72.7a	57.5b	1.2	<0.01	3.08	<0.01	<0.01	<0.01
0	48.5b	65.0a	2.4	0.01				
3	56.1	56.2	1.1	0.95				
9	89.0a	58.9b	1.6	<0.01				
13	97.8a	60.2b	6.4	0.01				
21	76.6a	52.9b	2.0	<0.01				
24	67.5a	51.6b	2.4	0.01				
Acetic acid (mM)								
Overall	51.3a	41.8b	1.13	<0.01	2.77	<0.01	<0.01	<0.01
0	36.9b	45.2a	1.82	0.03				
3	40.5	40.4	0.73	0.91				
9	52.3a	43.8b	1.34	<0.01				
13	67.1a	44.4b	4.81	0.03				
21	53.2a	39.1b	1.57	<0.01				
24	47.6a	38.1b	1.87	0.02				
Propionic acid (mM)								
Overall	13.8a	10.3b	0.19	<0.01	0.47	<0.01	<0.01	<0.01
0	8.4b	12.1a	0.24	<0.01				
3	10.1	9.9	0.20	0.60				
9	17.7a	10.1b	0.67	<0.01				
13	19.7a	10.8b	0.62	<0.01				
21	14.7a	9.5b	0.71	0.01				
24	12.4a	9.1b	0.50	0.01				
Acetic acid: propionic acid ratio								
Overall	3.8	4.1	0.11	0.11	0.26	0.11	0.25	<0.01
0	4.4a	3.7b	0.13	0.03				
3	4.0	4.1	0.06	0.59				
9	3.6b	4.4a	0.19	0.04				
13	3.4	4.1	0.22	0.09				
21	3.6	4.2	0.20	0.13				
24	3.8	4.2	0.12	0.10				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.

Table A6 Effect of alternate-day feeding of low protein concentrate on butyric acid, isobutyric acid, valeric acid and isovaleric acid concentrations

Hours after feeding	Concentrate fed	Concentrate not fed	SE[Z]	P[Y]	Repeated measures analysis			
					SE	Probability of diet	Probability of time	Probability of treatment x time
Butyric acid (mM)								
Overall	5.6a	3.7b	0.23	<0.01	0.57	<0.01	<0.01	<0.01
0	2.8b	5.4a	0.35	0.01				
3	3.8	4.0	0.20	0.57				
9	6.7a	3.4b	0.32	<0.01				
13	8.4a	3.6b	1.06	0.03				
21	6.4a	3.1b	0.24	<0.01				
24	5.3a	3.0b	0.23	<0.01				
Isobutyric acid (mMl)								
Overall	0.60	0.55	0.02	0.14	0.05	0.15	0.87	0.04
0	0.48	0.70	0.07	0.10				
3	0.52	0.56	0.04	0.54				
9	0.60	0.52	0.03	0.10				
13	0.68a	0.50b	0.04	0.04				
21	0.68	0.50	0.08	0.18				
24	0.64	0.52	0.06	0.24				
Valeric acid (mM)								
Overall	0.61a	0.38b	0.02	<0.01	0.06	<0.01	0.01	<0.01
0	0.24	0.60	0.05	0.10				
3	0.50	0.50	0.04	1.00				
9	0.80a	0.40b	0.07	0.02				
13	0.86a	0.32b	0.09	0.01				
21	0.70a	0.24b	0.04	<0.01				
24	0.58a	0.22b	0.02	<0.01				
Isovaleric acid (mM)								
Overall	0.81a	0.67b	0.03	0.02	0.07	0.02	0.24	<0.01
0	0.58b	0.96a	0.09	0.04				
3	0.62	0.78	0.06	0.14				
9	0.78a	0.54b	0.04	0.02				
13	1.04a	0.56b	0.08	0.01				
21	0.98a	0.58b	0.05	<0.01				
24	0.84a	0.60b	0.04	0.02				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.

Table A7 Effect of alternate-day feeding of high protein concentrate on rumen pH, ammonia, and lactic acid concentrations

Hours after feeding	Concentrate fed	Concentrate not fed	SE[Z]	P[Y]	Repeated measures analysis			
					SE	Probability of diet	Probability of time	Probability of treatment x time
Rumen ammonia concentration (mM)								
Overall	3.8a	2.2b	0.36	0.04	0.92	0.04	<0.01	<0.01
0	1.5b	3.1a	0.34	0.03				
3	11.8a	3.4b	1.80	0.03				
9	2.3	2.3	0.44	0.96				
13	1.3	0.9	0.14	0.14				
21	2.7	1.5	0.33	0.06				
24	3.1	1.7	0.35	0.06				
pH								
Overall	6.72b	7.07a	0.02	<0.01	0.06	<0.01	<0.01	<0.01
0	7.18a	6.99b	0.01	<0.01				
3	6.96b	7.08a	0.01	<0.01				
9	6.20b	7.03a	0.09	<0.01				
13	6.29b	6.99a	0.04	<0.01				
21	6.78b	7.12a	0.04	<0.01				
24	6.93b	7.23a	0.02	<0.01				
Lactic acid (mM)								
Overall	0.24	0.13	0.04	0.11	0.09	0.11	0.12	0.16
0	0.05	0.15	0.04	0.15				
3	0.30	0.17	0.11	0.45				
9	0.43	0.13	0.11	0.13				
13	0.30	0.14	0.06	0.15				
21	0.19	0.09	0.05	0.24				
24	0.16	0.09	0.02	0.12				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.

Table A8 Effect of alternate-day feeding of high protein concentrate on total volatile acid, acetic acid and propionic acid concentrations and acetic acid to propionic acid ratios

Hours after feeding	Concentrate fed	Concentrate not fed	SE[Z]	P[Y]	Repeated measures analysis			
					SE	Probability of diet	Probability of time	Probability of treatment x time
Total volatile fatty acids (mM)								
Overall	75.8a	54.5b	1.39	<0.01	3.41	<0.01	<0.01	<0.01
0	48.9b	66.0a	2.22	0.01				
3	59.3	54.4	3.32	0.36				
9	103.0a	54.0b	4.18	<0.01				
13	100.2a	56.1b	2.68	<0.01				
21	76.8a	51.2b	3.63	0.01				
24	66.6a	45.1b	2.58	<0.01				
Acetic acid (mM)								
Overall	53.4a	39.8b	0.89	<0.01	2.17	<0.01	<0.01	<0.01
0	36.2b	46.2a	1.65	0.01				
3	42.2	39.7	2.42	0.50				
9	72.6a	40.4b	2.74	<0.01				
13	69.0a	41.7b	1.27	<0.01				
21	53.4a	37.6b	2.74	0.02				
24	46.7a	33.3b	2.06	0.01				
Propionic acid (mM)								
Overall	14.5a	9.6b	0.44	<0.01	1.08	<0.01	<0.01	<0.01
0	8.6b	12.4a	0.36	<0.01				
3	11.0	9.5	0.54	0.13				
9	13.7a	9.1b	0.93	<0.01				
13	20.3a	9.9b	1.07	<0.01				
21	14.9a	9.0b	0.71	<0.01				
24	12.6a	7.8b	0.46	<0.01				
Acetic acid:propionic acid ratio								
Overall	3.8b	4.2a	0.10	0.04	0.24	0.04	0.45	<0.01
0	4.3a	3.7b	0.13	0.04				
3	3.8b	4.2a	0.08	0.04				
9	3.7b	4.5a	0.19	0.05				
13	3.4b	4.3a	0.20	0.04				
21	3.6b	4.2a	0.14	0.04				
24	3.7b	4.3a	0.13	0.04				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.

Table A9 Effect of alternate-day feeding of high protein concentrate on butyric, isobutyric, valeric and isovaleric concentrations

Hours after feeding	Concentrate fed	Concentrate not fed	SE[Z]	P[Y]	Repeated measures analysis			
					SE	Probability of diet	Probability of time	Probability of treatment x time
Butyric acid (mM)								
Overall	5.9a	3.3b	0.25	<0.01	0.62	<0.01	<0.01	<0.01
0	2.8b	4.8a	0.29	0.01				
3	4.5	3.4	0.33	0.08				
9	8.3a	3.0b	0.77	0.01				
13	8.5a	3.2b	0.54	<0.01				
21	6.1a	3.1b	0.29	<0.01				
24	5.0a	2.6b	0.19	<0.01				
Isobutyric acid (mM)								
Overall	0.57	0.57	0.01	0.49	0.03	1.00	0.51	<0.01
0	0.45b	0.78a	0.07	0.03				
3	0.46b	0.66a	0.05	0.05				
9	0.54	0.50	0.05	0.59				
13	0.62a	0.50b	0.03	0.03				
21	0.68	0.50	0.05	0.07				
24	0.68a	0.50b	0.04	0.04				
Valeric acid (mM)								
Overall	0.66a	0.35b	0.03	<0.01	0.08	<0.01	<0.01	<0.01
0	0.20b	0.60a	0.07	0.02				
3	0.60a	0.42b	0.03	0.02				
9	1.02a	0.32b	0.09	0.01				
13	0.88a	0.30b	0.05	<0.01				
21	0.70a	0.24b	0.05	<0.01				
24	0.54a	0.20b	0.03	<0.01				
Isovaleric acid (mM)								
Overall	0.83a	0.74b	0.02	0.03	0.04	0.03	0.03	<0.01
0	0.64b	1.24a	0.14	0.04				
3	0.60b	0.82a	0.05	0.04				
9	0.78	0.56	0.07	0.10				
13	0.96a	0.52b	0.02	<0.01				
21	1.00a	0.66b	0.04	<0.01				
24	0.98a	0.64b	0.06	0.02				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.

Table A10 Effect of every third day feeding of high protein concentrate on rumen pH, ammonia, and lactic acid concentrations

Hours after feeding	Concentrate fed					Repeated measures analysis			
	On this day	1 day previous	2 days previous	SE[Z]	P[Y]	SE	Probability of diet	Probability of time	Probability of treatment x time
Rumen ammonia concentration (mM)									
Overall	5.1a	2.6b	2.1b	0.59	0.01	1.44	0.01	<0.01	<0.01
0	1.6	3.1	2.3	0.55	0.24				
3	11.2a	3.9b	3.9b	1.33	0.01				
9	4.7a	2.6b	1.4b	0.49	<0.01				
13	5.1	1.5	1.3	1.14	0.08				
21	4.3	2.4	1.7	0.74	0.10				
24	3.7	2.4	1.9	0.61	0.15				
pH									
Overall	6.63b	6.93a	7.03a	0.06	<0.01	0.16	<0.01	<0.01	<0.01
0	7.21a	6.78c	7.10b	0.03	<0.01				
3	7.03a	6.88b	7.10a	0.03	0.01				
9	6.21	6.92	6.90	0.20	0.06				
13	5.96b	6.82a	6.81a	0.14	<0.01				
21	6.63b	7.04a	7.12a	0.07	<0.01				
24	6.76b	7.12a	7.15a	0.04	<0.01				
Lactic acid (mM)									
Overall	0.82	0.12	0.10	0.30	0.22	0.75	0.22	0.40	0.43
0	0.18	0.17	0.10	0.06	0.55				
3	0.39	0.14	0.10	0.08	0.07				
9	3.09	0.09	0.08	1.65	0.37				
13	0.82a	0.10b	0.12b	0.17	0.02				
21	0.29	0.10	0.09	0.09	0.23				
24	0.12	0.09	0.10	0.10	0.43				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.

Table A11 Effect of every third day feeding of high protein concentrate on total volatile fatty acids, acetic acid and propionic acid concentrations and acetic acid to propionic acid ratios

Hours after feeding	Concentrate fed					Repeated measures analysis			
	On this day	1 day previous	2 days previous	SE[Z]	P[Y]	SE	Probability of diet	Probability of time	Probability of treatment x time
Total volatile fatty acids (mM)									
Overall	77.4a	65.5ab	56.3b	4.45	0.03	10.9	0.03	<0.01	<0.01
0	42.2c	76.9a	60.7b	4.40	<0.01				
3	59.5b	67.5a	50.9c	2.38	<0.01				
9	94.3a	66.5ab	57.6b	9.02	0.05				
13	105.4a	71.8b	62.5b	7.97	0.01				
21	83.9a	58.7b	54.3b	5.80	0.01				
24	78.5a	51.8b	51.7b	4.91	0.01				
Acetic acid (mM)									
Overall	52.4	46.5	41.0	2.92	0.07	7.15	0.07	<0.01	<0.01
0	31.5b	51.7a	43.9a	3.09	<0.01				
3	43.0ab	47.1a	37.4b	1.86	0.02				
9	64.2	48.4	42.5	6.27	0.10				
13	68.7a	51.7ab	45.3b	5.58	0.04				
21	54.8a	42.4b	39.4b	3.70	0.04				
24	52.2a	37.4b	37.7b	2.97	0.01				
Propionic acid (mM)									
Overall	15.2a	12.5b	10.2b	0.78	<0.01	1.92	0.01	<0.01	<0.01
0	7.2c	15.4a	10.9b	0.83	<0.01				
3	11.1b	12.9a	8.9c	0.47	<0.01				
9	19.2a	12.2b	10.3b	1.00	<0.01				
13	21.3a	13.7b	11.7b	1.47	<0.01				
21	16.8a	11.0b	9.9b	1.09	<0.01				
24	15.6a	9.5b	9.3b	1.02	<0.01				
Acetic acid to propionic acid ratio									
Overall	3.6b	3.8b	4.1a	0.06	<0.01	0.14	<0.01	0.04	<0.01
0	4.4a	3.4c	4.0b	0.10	<0.01				
3	3.9ab	3.7b	4.2a	0.11	0.02				
9	3.3b	3.9a	4.2a	0.14	<0.01				
13	3.2b	3.8a	4.1a	0.16	<0.01				
21	3.2b	3.8a	4.1a	0.08	<0.01				
24	3.4b	3.9a	4.1a	0.09	<0.01				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.

Table A12 Effect of every third day feeding of high protein concentrate on butyric, isobutyric, valeric and isovaleric acid concentrations

Hours after feeding	Concentrate fed					Repeated measures analysis			
	On this day	1 day previous	2 days previous	SE[Z]	P[Y]	SE	Probability of diet	Probability of time	Probability of treatment x time
Butyric acid (mM)									
Overall	7.8a	4.5b	3.6b	0.70	<0.01	1.71	0.01	<0.01	<0.01
0	2.5b	7.3a	4.0b	0.50	<0.01				
3	4.3b	5.1a	2.9c	0.19	<0.01				
9	9.1a	3.9b	3.6b	1.35	<0.01				
13	13.3a	4.4b	4.2b	1.23	<0.01				
21	9.6a	3.4b	3.5b	0.94	<0.01				
24	7.9a	3.0b	3.3b	0.72	<0.01				
Isobutyric acid (mM)									
Overall	0.49b	0.70a	0.51b	0.04	0.01	0.09	0.01	<0.01	<0.01
0	0.40b	0.76a	0.66a	0.07	0.02				
3	0.34c	0.70a	0.56b	0.04	<0.01				
9	0.36b	0.64a	0.42b	0.05	0.01				
13	0.48b	0.72a	0.42b	0.05	0.01				
21	0.66	0.72	0.50	0.07	0.11				
24	0.72a	0.64ab	0.50b	0.05	0.05				
Valeric acid (mM)									
Overall	0.74a	0.52ab	0.37b	0.07	0.02	0.17	0.02	0.01	<0.01
0	0.16c	0.78a	0.38b	0.06	<0.01				
3	0.46b	0.66a	0.40b	0.04	<0.01				
9	0.96a	0.48b	0.38b	0.09	<0.01				
13	1.00a	0.46b	0.44b	0.12	0.01				
21	0.98a	0.38b	0.36b	0.11	0.01				
24	0.90a	0.34b	0.28b	0.11	0.01				
Isovaleric acid (mM)									
Overall	0.67b	0.80a	0.61b	0.06	0.03	0.14	0.03	<0.01	<0.01
0	0.46b	0.98a	0.90a	0.08	<0.01				
3	0.42c	0.94a	0.66b	0.07	<0.01				
9	0.44b	0.74a	0.42b	0.07	0.02				
13	0.62ab	0.82a	0.44b	0.06	0.01				
21	1.02	0.86	0.62	0.11	0.09				
24	1.04	0.82	0.64	0.13	0.17				

[Z] Standard error is based upon five animals per mean with samples obtained on each of two days at each time.
[Y] Probability of difference.
a,b Means not followed by the same letter differ.